VOLUME 30 JULY 1952 NUMBER 7

Canadian Journal of Chemistry

Editor: J. W. T. SPINKS

Published by THE NATIONAL RESEARCH COUNCIL
OTTAWA CANADA

CANADIAN JOURNAL OF CHEMISTRY

(Formerly Section B, Canadian Journal of Research)

The CANADIAN JOURNAL OF CHEMISTRY is published monthly by the National Research Council of Canada under the authority of the Chairman of the Committee of the Privy Council on Scientific and Industrial Research. Matters of general policy are the responsibility of a joint Editorial Board consisting of members of the National Research Council of Canada and the Royal Society of Canada.

The National Research Council of Canada publishes also: *Canadian Journal of Botany, Canadian Journal of Medical Sciences, Canadian Journal of Physics, Canadian Journal of Technology, Canadian Journal of Zoology.*

The CANADIAN JOURNAL OF CHEMISTRY and the CANADIAN JOURNAL OF TECHNOLOGY have been chosen by the Chemical Institute of Canada as its medium of publication for scientific papers.

EDITORIAL BOARD

Representing
NATIONAL RESEARCH COUNCIL

DR. J. H. L. JOHNSTONE (*Chairman*),
Professor of Physics,
Dalhousie University,
Halifax, N.S.

DR. OTTO MAASS,
Macdonald Professor of
Physical Chemistry,
McGill University,
Montreal, P.Q.

DR. CHARLES W. ARGUE,
Dean of Science,
University of New Brunswick,
Fredericton, N.B.

DR. A. G. MCCALLA,
Dean, Faculty of Agriculture
University of Alberta,
Edmonton, Alta.

Representing
ROYAL SOCIETY OF CANADA

DR. G. M. VOLKOFF,
Professor of Physics,
University of British Columbia,
Vancouver, B.C.

DR. J. W. T. SPINKS,
Dean, College of Graduate
Studies,
University of Saskatchewan,
Saskatoon, Sask.

} Section III

DR. H. S. JACKSON,*
Head, Department of Botany,
University of Toronto,
Toronto, Ont.

DR. E. HORNE CRAIGIE,
Department of Zoology,
University of Toronto,
Toronto, Ont.

} Section V

Ex officio

DR. LÉO MARION (*Editor-in-Chief*),
Director, Division of Pure Chemistry,
National Research Laboratories,
Ottawa.

DR. H. H. SAUNDERSON,
Director, Division of Information Services,
National Research Council,
Ottawa.

Representing
THE CHEMICAL INSTITUTE OF CANADA

DR. H. G. THODE,
Department of Chemistry
McMaster University,
Hamilton, Ont.

Manuscripts should be addressed to:

DR. LÉO MARION,
Editor-in-Chief,
Canadian Journal of Chemistry,
National Research Council,
Ottawa, Canada.

Each manuscript should be typewritten, double-spaced, and the original and one extra copy submitted (see **Notice to Contributors** inside of back cover).

Subscriptions, renewals, and orders for back numbers should be addressed to:

Administrative Services,
National Research Council,
Ottawa, Canada.

Subscription rate: $4.00 a year; single numbers: 50 cents. Special rates can be obtained for subscriptions to more than one of the Journals published by the National Research Council.

*Deceased.

Canadian Journal of Chemistry

Issued by THE NATIONAL RESEARCH COUNCIL OF CANADA

VOL. 30 JULY, 1952 NUMBER 7

THE REACTIONS OF ACETALDEHYDE WITH METHANOL[1]

BY G. W. MEADOWS[2] AND B. DEB DARWENT

ABSTRACT

The nature of the products obtained from reactions of acetaldehyde with methanol have been investigated in neutral and acidic solutions by titration with iodine and sodium bisulphite, ultraviolet absorption, and infrared spectroscopy. In neutral and buffered solution the hemiacetal is the only important product; in the presence of a high concentration of hydrogen ions the acetal is produced nearly quantitatively.

INTRODUCTION

It is well known that the reaction between many aldehydes and alcohols leads to the formation of acetals (1,4)

$$R.CHO + 2\,R'.OH \rightarrow R.CH\begin{matrix}OR'\\OR'\end{matrix} + H_2O \qquad (1)$$

and the reaction is believed to proceed through the formation of the corresponding hemiacetal: (1,4,6,8)

$$R.CHO + R'.OH \rightarrow R.CH\begin{matrix}OR'\\OH\end{matrix} \qquad (2)$$

Hemiacetals are usually unstable compounds and are therefore difficult to isolate, but their existence, in reasonable concentration, in solutions has been postulated in a number of cases. To interpret measurements of the rate of reaction of acetaldehyde with methanol (7), we have found it necessary to study in some detail the factors governing the formation of acetal and hemiacetal and to investigate the possible formation of other products, such as would be formed by condensation and polymerization reactions of acetaldehyde.

EXPERIMENTAL

The nature of the reactions that occur when acetaldehyde and methanol are mixed under different conditions was investigated by three methods—titration with bisulphite and iodine solutions, ultraviolet absorption, and infrared spectroscopy.

[1] *Manuscript received March 5, 1952.*
Contribution from the Division of Chemistry, National Research Laboratories, Ottawa, Canada. Issued as N.R.C. No. 2746.

[2] *National Research Council Postdoctoral Fellow 1948-50; Present address: c/o E. I. duPont de Nemours, Wilmington 98, Delaware.*

Materials

Acetaldehyde was supplied by the Eastman Kodak Company in sealed bottles and was kept in cold storage until required. Some of the samples used were distilled in an atmosphere of nitrogen and collected in small glass phials which were then sealed and used immediately after preparation. Reagent methanol, supplied by the Brickman Company, was used without further treatment. Phillips Petroleum Co.'s Research Grade *n*-heptane was dried with sodium before use. Analar Grade sodium chloride was heated to 150°C. for several hours and stored in an evacuated desiccator. Commercial hydrochloric acid, sp. gr. 1.188, was used in preparing the dilute solution employed.

Bisulphite Titration

The procedure was that described by Friedemann, Cotonio, and Shaffer (5). Ten cubic centimeters of the methanolic solution of acetaldehyde was added to 25 cc. of 0.092 *M* (excess) sodium bisulphite and allowed to stand with occasional shaking. The excess of bisulphite was titrated with standard iodine solution and the concentration of aldehyde calculated. This was checked by decomposing the bisulphite compound, by the addition of solid sodium bicarbonate, and by titrating the liberated bisulphite with standard iodine solution. It was found that, with "neutral" methanolic solutions,* reproducible results were obtained only if the solution was left for at least 30 min. for the first reaction to be completed. Under those conditions the concentrations of aldehyde as measured by the two methods agreed (within $\pm$ 0.5%) with that calculated from the initial concentration of aldehyde. On the other hand, if methanolic hydrochloric acid were added to the solution of acetaldehyde in methanol, the final concentration of hydrochloric acid being about 0.001*N*, and if sufficient time were allowed for the reaction to be completed, it was found that only 6% of the original acetaldehyde added was converted to the bisulphite compound after 30 min. However, when 10 cc. of the completely reacted solution of acetaldehyde in methanolic hydrochloric acid was added to 25 cc. of 1 *N* aqueous hydrochloric acid, heated to 60°C, cooled, and titrated, 60% of the acetaldehyde could be estimated as the bisulphite compound. In this latter case it was not feasible to estimate the excess of bisulphite, since some of it had decomposed in the strongly acid solution; but it was possible to estimate the acetaldehyde–bisulphite compound by neutralizing the excess of bisulphite with iodine, making alkaline with sodium bicarbonate to decompose the acetaldehyde–bisulphite compound, and then estimating the liberated bisulphite by further titration with iodine.

Products

The product obtained from the hydrochloric acid catalyzed reaction was isolated and purified. The bulk of the methanol was removed by shaking with excess calcium chloride and the solid mass which resulted was broken up and evacuated through a trap cooled to $-$ 80°C. The liquid condensate in the trap was treated with sodium until no further gas was evolved and distilled twice from calcium chloride, the final product being collected between 63.5°C. and

**These solutions contained only such acids as were present in the methanol and acetaldehyde.*

63.8°C. at 761 mm. The boiling point of dimethyl acetal has been reported as being between 63°C. and 65°C. (3,9); a sample supplied by the Eastman Kodak Company was found to have a boiling point of 63.4°C. at 758 mm.

Ultraviolet Absorption

Herold and Wolf (6) studied the reaction between various aldehydes and alcohols spectrometrically. We have examined the acetaldehyde methanol system by this method, using Beckman Model D.U. spectrophotometer with 1 cm. quartz cells. The absorption spectra of *n*-heptane solutions of untreated acetaldehyde (0.15 *M*) and of acetaldehyde distilled under nitrogen (0.15 *M*) were measured over the range λ 2200 Å to 2300 Å. The absorption maxima and extinction coefficients are recorded in Table I together with values previously reported in the literature.

TABLE I

ABSORPTION COEFFICIENT OF ACETALDEHYDE IN *n*-HEPTANE

	$\lambda_{max.}$, Å	log $\epsilon_{max.}$
Untreated acetaldehyde	2900	1.19
Distilled acetaldehyde	2900	1.22
Bielecki and Henri (2)	2900	0.79
Schou (10)	2950	1.07
Herold and Wolf (6) (unpurified)	2900	1.07
Herold and Wolf (6) (purified)	2900	1.23

The lower values obtained for log $\epsilon_{max.}$ are probably caused by the presence of polymer in the untreated acetaldehyde and the agreement obtained with Herold and Wolf's figure suggests that the nitrogen distilled acetaldehyde used in our experiments was reasonably pure.

The rate of the reaction of acetaldehyde with methanol was measured spectrometrically. The solution was prepared by breaking a glass phial containing a known weight of acetaldehyde under a known volume of methanol. After the solution was stirred for one minute a 1 cm. quartz cell was filled and the optical density measured at frequent intervals, the instrument being set at λ2925 Å. The results of two experiments, 299 and 301, are shown in Fig. 1; experiment No. 299 represents the disappearance of the carbonyl band in neutral solution and No. 301 the subsequent reaction on addition of acid.The plot of log $(d_t - d_\infty)$ for experiment 299 was approximately linear during the early stages but later a slight curvature occurred, indicating a small acceleration in rate. The temperature of the solution was found to have increased from 25° to 31.5°C. during the course of the reaction, probably owing to the heating effect of the adjacent lamp housing, and it is likely that this was responsible for the increased rate in the latter part of the experiment. The rate constants at the start and end of the reaction were 5.42×10^{-2} and 6.66×10^{-2} min.$^{-1}$ Extrapolation of the initial linear portion to zero time showed the initial density to be 3.8, which corresponds to log $k_{max.} = 1.22$ for the 0.228 *M* solution. This value, in conjunction with the final density value of 0.208, showed that 95% of the original material had reacted. It is interesting to note

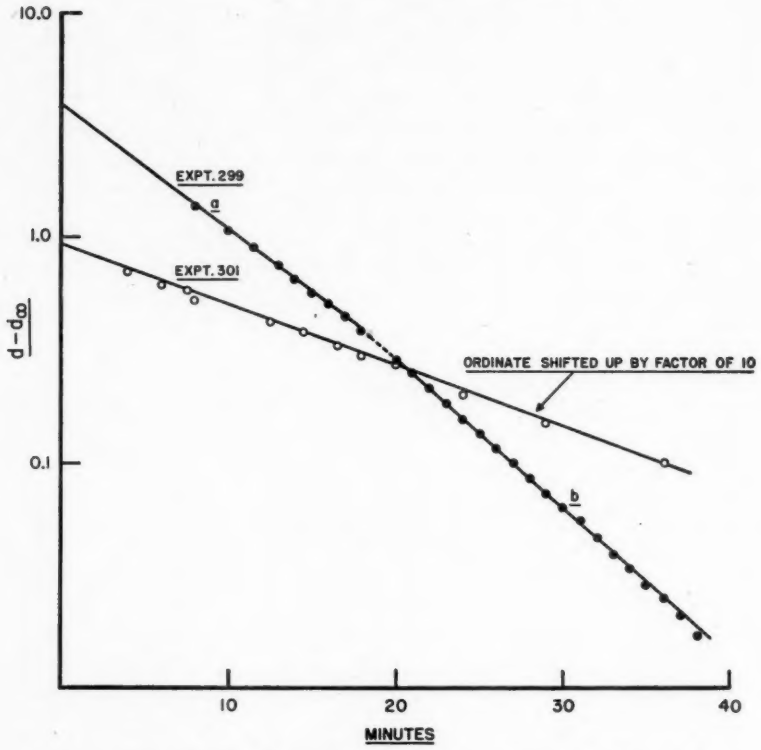


FIG. 1. The effect of time on the intensity of the carbonyl absorption at 2925 Å, of a solution of acetaldehyde in methanol at "room temperature".
Expt. 299—neutral solution; acetaldehyde concentration (initial) = 0.228 *M*.
Expt. 301—reaction induced by adding 1 cc. of 0.114 *N* hydrochloric acid to 25 cc. of the completely reacted neutral solution from Expt. 299.

that the initial molar extinction coefficient of acetaldehyde was the same in methanol as in *n*-heptane.

The reaction which occurs when 1 cc. of 0.114 *N* hydrochloric acid is added to 25 cc. of a reacted solution of acetaldehyde in neutral methanol has also been followed spectrometrically (Fig. 1, Expt. 301). It was found to correspond to the complete disappearance of the carbonyl group remaining in solution and to proceed in a first order manner. The velocity constant for this reaction was 2.70×10^{-2} min.$^{-1}$

Infrared Spectroscopy

The products of the uncatalyzed and hydrochloric acid catalyzed reactions were characterized by infrared absorption measurements,* using methanol as the solvent. The strong absorption of methanol made it necessary to use a very

**We are indebted to Dr. R. N. Jones for the use of the spectrometer and to Dr. A. R. H. Cole and Mr. R. Lauzon for the measurements.*

thin cell and, therefore, somewhat higher concentrations of acetaldehyde than in the dilatometer experiments. The solutions were between 0.80 *M* and 0.85 *M* and the measurements made with a Perkin-Elmer 12 C Infrared Spectrometer with a rock salt prism and a 0.025 mm. cell. The absorption curves between 880 cm.$^{-1}$ and 1240 cm.$^{-1}$, for two solutions of the same concentrations, one containing the purified product of the hydrochloric acid catalyzed reaction of acetaldehyde and methanol and the other Eastman Kodak dimethyl acetal in methanol, were found to be identical (Curve *A*, Fig. 2).

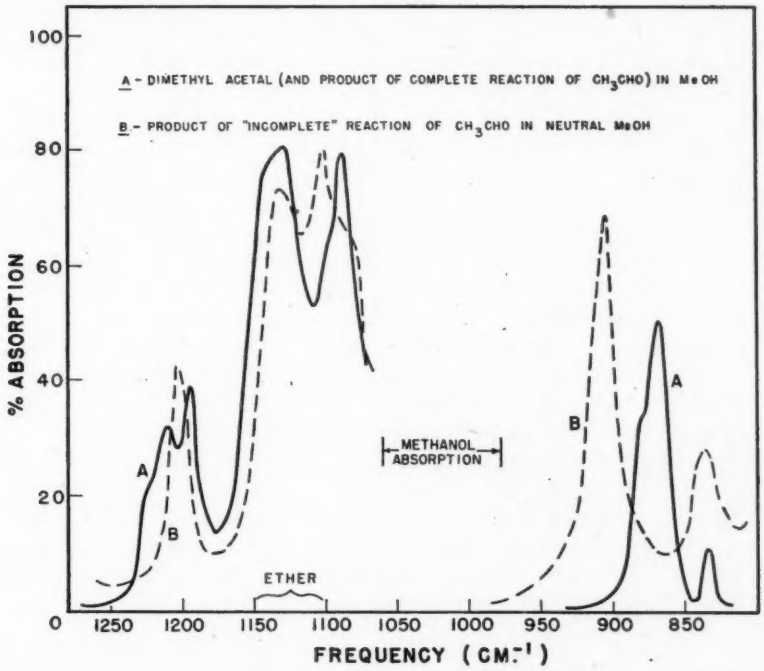


FIG. 2. Infrared absorption spectra of the products of the reaction of acetaldehyde with methanol.

A 0.853 *M* solution of acetaldehyde was prepared in 0.0016 *N* methanolic hydrochloric acid. After sufficient time was allowed for the reaction to be completed, the infrared absorption, measured over the same range, was found to be identical with that observed for the two solutions previously examined. For a 0.867 *M* solution of dimethyl acetal in methanol the optical density at 869 cm.$^{-1}$ was found to be 0.292. The optical density of the reacted acetaldehyde solution at 869 cm.$^{-1}$ was 0.285, corresponding to concentrations of 0.847 *M*, which further supports the conclusion that the acetal formation reaction goes nearly to completion in the presence of excess methanol.

A 0.80 *M* solution of acetaldehyde in neutral methanol was prepared and

allowed to react completely. The spectrum was measured in the region 880 cm.$^{-1}$ to 1240 cm.$^{-1}$ and the resulting curve (Curve *B*, Fig. 2) was found to be quite different from that obtained for the acetal solutions. It is not possible to correlate the observed spectrum with that of methyl hemiacetal, since this substance cannot be obtained in the form of a pure stable compound. However, the spectrum of the intermediate product showed strong absorption at 1140 cm.$^{-1}$, indicating an ether linkage, as expected if the hemiacetal were produced. The observed difference between the two curves shows that unique products are formed at each stage of the reaction between methanol and acetaldehyde.

CONCLUSIONS

The results given above indicate strongly that the hydrochloric acid catalyzed reaction of acetaldehyde with methanol results in the formation of acetaldehyde dimethyl acetal and that no other products such as aldol or polymers are produced in significant amounts. Ultraviolet spectroscopy showed that the carbonyl group completely disappears, infrared spectroscopy showed that the only detectable product had a spectrum indistinguishable from that of dimethylacetal, and the bisulphite titration showed that the compound produced did not react readily to form the bisulphite addition compound. This latter evidence rules out the possibility of the production of paraldehyde.

In "neutral methanol" it is probable that acetaldehyde forms only the hemiacetal. This was shown by the fact that all of the acetaldehyde could be accounted for as the bisulphite compound, that the intensity of the carbonyl band at 2900 Å was greatly diminished, and by the fact that the compound formed had an infrared spectrum quite different from that of the dimethylacetal and showed a strong absorption at 1140 cm.$^{-1}$, which is characteristic of the ether linkage.

Hence the two reactions (1) and (2) leading to the hemiacetal and the acetal may be readily separated and studied individually by controlling the hydrogen ion concentration, also the system is not complicated by the occurrence of other reactions such as the formation of aldols or polymers.

REFERENCES

1. Adkins, H. and Broderick, A. E. J. Am. Chem. Soc. 50: 178; 499. 1928.
2. Bielecki, J. and Henri, V. Ber. 46: 3627. 1913.
3. Claus, Ad. and Trainer, E. Ber. 19: 3004. 1886.
4. De Leeuw, H. L. Z. physik. Chem. 77: 284. 1911.
5. Friedemann, T. E., Cotonio, M., and Shaffer, P. A. J. Biol Chem. 73: 342. 1927.
6. Herold, W. and Wolf, K. L. Z. physik. Chem., B, 12: 165. 1931.
7. Meadows, G. W. and Darwent, B.deB. (In press.)
8. Melchoir, N. C. J. Am. Chem. Soc. 71: 3651. 1949.
9. Schiff, R. Ann. 220: 104. 1883.
10. Schou, S. A. J. chim. phys. 26: 77. 1929.

EFFECT OF ADDITION AGENTS ON COPPER DEPOSITION IN A CONVECTION-FREE SYSTEM[1]

By R. C. Turner and C. A. Winkler

ABSTRACT

The effects of gelatin and chloride ion on deposition in a convection-free system were similar in most respects to those observed when convection currents were present. Gelatin concentrations up to and including 250 mgm. per liter and chloride concentrations of 4 and 45 mgm. per liter had no effect on concentration polarization. There was a difference, however, in the effect of the two addition agents on polarization other than concentration polarization.

INTRODUCTION

It was shown in an earlier paper (11) that concentration polarization during copper deposition could be distinguished from other kinds of polarization by using a special type of Haring cell which effectively eliminated convection currents near the cathode. Studies previously reported in the literature on the effect of addition agents on copper deposition have all been made with vertical cathodes and consequently in the presence of convection currents. It was therefore decided to make some experiments with the cell mentioned above to determine whether the elimination of convection currents altered in any way the action of gelatin and chloride ion in copper deposition, and to determine the effect of these addition agents on the polarization under convection-free conditions.

Although adsorption appears to play an important part in the action of many addition agents (1, 2, 4, 10), there seems to be little direct information on the effect of a layer of adsorbed addition agent on copper deposition, apart from the observation that such a layer decreased adhesion of the deposit (5). Some attention has also been given to this aspect of the problem in the present study.

EXPERIMENTAL

The vertical type Haring cell used has been described in detail (11). In brief, it consisted of a cylindrical cell of which the copper cathode and anode formed respectively the top and bottom. Two nonworking electrodes of copper screens divided the cell into three compartments of equal lengths. The cathode was carefully leveled for each experiment, and the polarization values determined as in the previous study.

For some of the experiments the addition agents, gelatin and chloride ion (as sodium chloride), were added singly or in combination directly to the standard electrolyte, which consisted of 125 gm. copper sulphate pentahydrate and 100 gm. concentrated sulphuric acid per liter of redistilled water. In other experiments the cathode was suspended for 20 min. in 100 ml. of stirred electrolyte containing various quantities of the addition agents. The

[1] *Manuscript received December 21, 1951.*
Contribution from the Physical Chemistry Laboratory, McGill University, Montreal, Que. and the Division of Chemistry, Science Service, Canada Department of Agriculture, Ottawa. Scientific Contribution No. 216, Division of Chemistry, Science Service.

cathode was then washed with a stream of redistilled water and replaced in the cell, which for these experiments contained a 10-fold dilution of the standard electrolyte.

When the addition agents were added directly to the standard electrolyte, an apparent current density of 6.0×10^{-3} amp. per cm.^{2} was used, while in the other experiments with the diluted electrolyte the apparent current density was 6.72×10^{-4} amp. per cm.^{2}

RESULTS AND DISCUSSION

(*a*) *Effect of Addition Agents Added to the Cell Electrolyte*

The polarization–time curves for various concentrations of gelatin in the electrolyte (Fig. 1) show that the time required to reach the rapid increase in

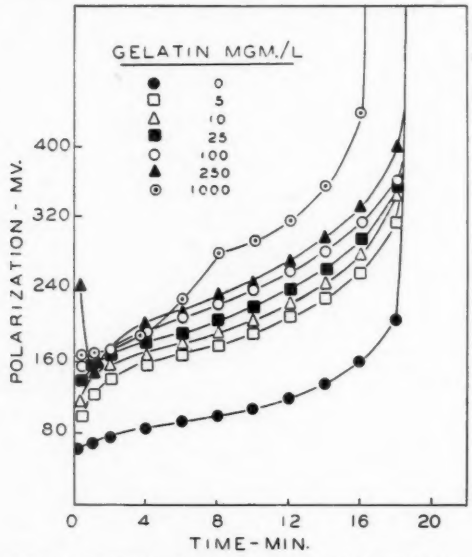


FIG. 1. Effect of gelatin in the electrolyte on polarization–time curves.

polarization was the same with or without gelatin in the electrolyte, except when the concentration was 1000 mgm. per liter. This means that gelatin had no measurable effect on concentration polarization (11) with concentrations up to and including 250 mgm. per liter. With concentrations above 2 mgm. per liter, polarization decreased very rapidly within the first half minute, after which it increased again in the same general way as in the absence of addition agents. The initial decrease was so rapid (Fig. 1, 250 mgm. per liter) that it was generally impossible to determine polarization values until after one-half minute of electrolysis. As with vertical cathodes (1, 2, 4, 10), relative changes in polarization values were most pronounced with low concentrations of gelatin (0-5 mgm. per liter) which indicates that adsorption of the addition agent is involved.

The cathode surfaces after electrolysis in the presence of gelatin were glassy smooth and retained the color of the initial surface. Thus, when the initial surface was pale salmon-colored, the surface remained this color after 40 min. of electrolysis in the presence of gelatin. On the other hand, when the initially bright surface was rubbed with No. 2 emery cloth, the scratched bright appearance remained after electrolysis. These results differ from those obtained with vertical cathodes (6, 10) where addition agents often cause striations in the deposits.

Sodium chloride alone in the electrolyte caused some increase in cathode polarization (Fig. 2) but the effect was generally less than with gelatin. The

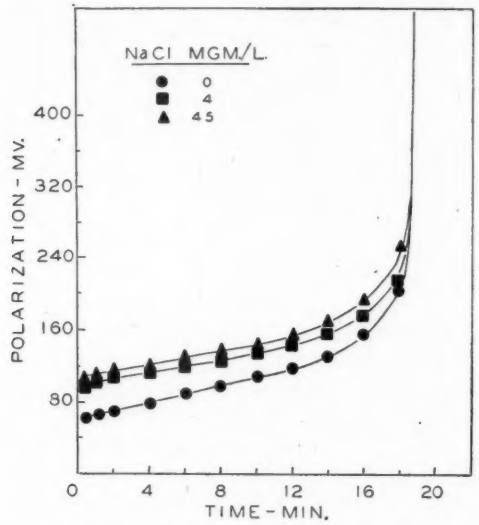


FIG. 2. Effect of sodium chloride in the electrolyte on polarization curves.

chloride caused only a slight darkening of the initial salmon-colored surface and, as with gelatin, there was a complete absence of striations.

In the experiments in which gelatin and sodium chloride were added together to the electrolyte, the gelatin concentration was kept constant at 25 mgm. per liter while the sodium chloride was varied from 1 to 87 mgm. per liter. When the chloride concentration was greater than 6 mgm. per liter the cathode surface was rough and rust colored after electrolysis, while when the concentration was below 3 mgm. per liter the deposited surface had the same appearance as with gelatin alone. With concentrations of 3, 4, and 6 mgm. per liter the deposits were partly smooth and partly rusty red, which gave the deposit a mottled appearance. As shown in Fig. 3, where some of the results are presented, the polarization values during most of electrolysis decreased as the sodium chloride concentration was increased up to 2 mgm. per liter, and then increased with increasing concentrations up to 6 mgm. per liter, after which they

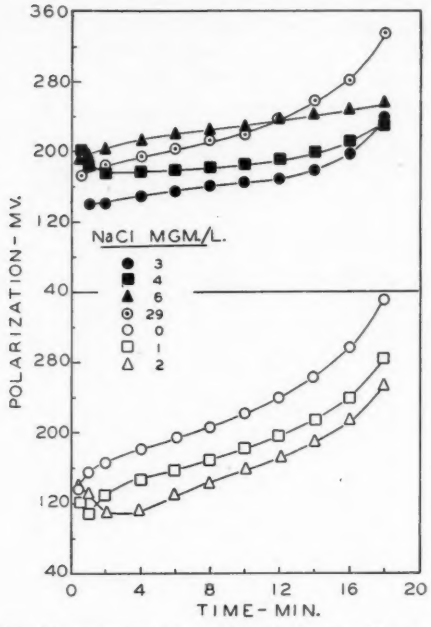


FIG. 3. Effect of chloride concentration on the polarization–time curves with 25 mgm. per liter of gelatin in the electrolyte.

approached those for gelatin alone. Thus a minimum in the polarization–chloride concentration curve was obtained, similar to that found by McConnell *et al.* (6). The slopes of the curves in Fig. 3 for sodium chloride concentrations of 3, 4, and 6 mgm. per liter were considerably less than for the other concentrations, probably as a result of surface changes during electrolysis (mottled appearance mentioned above).

(*b*) *Initial Adsorption of Addition Agents*

Study of the effect of adsorbed addition agent on the surface of the cathode was prompted by the observation that, during the experiments with gelatin and gelatin plus chloride, the polarization values appeared to start at a very high value and then decreased very rapidly during the first 15 to 30 sec. (Fig. 1, 250 mgm. per liter). The current density was reduced to 6.72×10^{-4} amp. per cm.^{2} to prolong as much as possible the effect of the adsorbed substances.

From the results in Fig. 4. which were obtained with a salmon-colored deposited surface, it appeared that adsorption occurred both from solutions containing gelatin alone and from solutions containing gelatin plus chloride, and caused a high initial polarization which decreased rapidly during the first two minutes. After the rapid decrease the polarization–time curves were above but more or less parallel to the curves obtained when the cathode, prior to electrolysis, was immersed in electrolyte containing no addition agent. Sodium chloride alone was apparently not adsorbed.

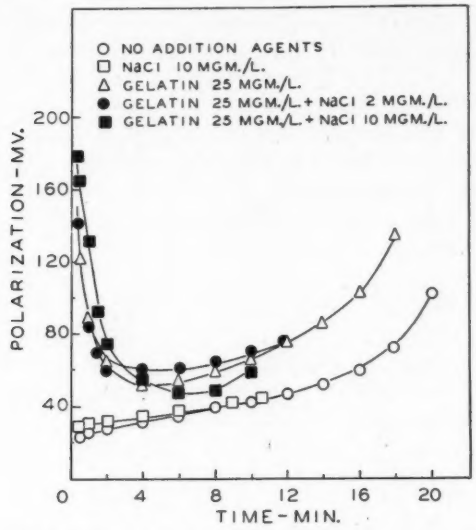


FIG. 4. Adsorption of addition agents as shown by polarization studies with salmon-colored deposited surfaces.

The high initial polarization was probably caused by an adsorbed layer of gelatin. The fact that this initially high polarization occurred as well when gelatin was present in the electrolyte (Part (*a*) above) indicates that an adsorbed layer of gelatin was formed in the interval of time between assembling the cell and the beginning of electrolysis. The rapid decrease in polarization may then correspond to incorporation of the gelatin into the deposited copper. The higher polarization values during most of electrolysis, relative to those occurring in the complete absence of gelatin, were not entirely the result of continuous adsorption during electrolysis because they occurred when the electrolyte was free from gelatin. Consequently, it was concluded that the type of surface caused by the presence of the gelatin plays an important part in cathode polarization.

The deposits in the presence of adsorbed gelatin plus chloride were always rust colored. The change from the initial salmon-colored surface commenced soon after electrolysis began, as indicated by an experiment in which the current was broken about two minutes after electrolysis started.

(*c*) *The Effect of Addition Agents on the Shape of the Polarization–Time Curves*

If the discharge of hydrated ions to form neutral atoms is the rate-governing step at a cathode, the relation between current density and polarization may be expressed by an equation (3, 7, 12) of which one form is (11)

$$\Delta E = \frac{RT}{azF} \ln \frac{C_0}{C_e} + \frac{RT}{azF} \ln \left(\frac{i}{I_0} + e^{-(1-a)zF\Delta E/RT} \right). \tag{1}$$

In this equation, I_0 represents the current flowing in either direction at equili-

brium, α is a constant with a value between zero and unity, C_0 and C_e are respectively the concentrations* in the body of the solution and at the electrode, R, T, and F have their usual meanings, and z is the number of electrons per ion involved in the electrode reaction.

In a convection free system, C_e is a function of duration of electrolysis, t, and it can be shown (8,9) that $RT/zF \ln(C_0/C_e)$ is equal to $-0.030 \log (1 - \sqrt{t}/\sqrt{\tau})$, where τ is the time required to reach the rapid increase in polarization, i.e., the time for C_e to decrease from concentration C_0 to essentially zero. Equation (1) may then be written

$$(2) \quad \Delta E = -\frac{0.030}{\alpha} \log \left(1 - \frac{\sqrt{t}}{\sqrt{\tau}}\right) + \frac{0.030}{\alpha} \log \left(\frac{i}{I_0} + e^{-(1-\alpha)zF\Delta E/RT}\right).$$

If i is much larger than I_0 and ΔE is large, the second term on the right-hand side of Equation (2) is practically equal to $0.030/\alpha \log (i/I_0)$, a constant providing the condition of the cathode surface does not change during electrolysis. Under these conditions, the plot of ΔE against $-\log (1 - \sqrt{t}/\sqrt{\tau})$

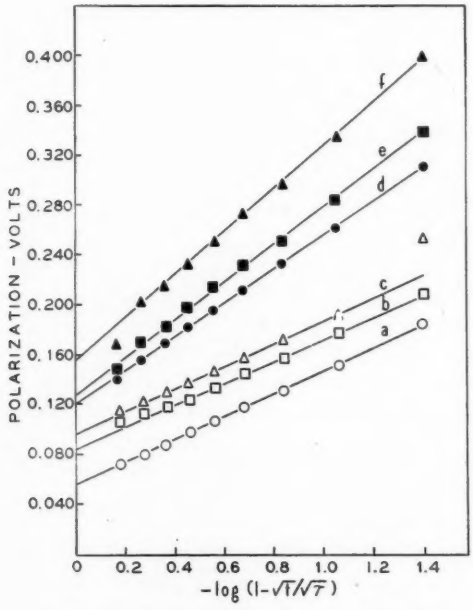


FIG. 5. Polarization vs $-\log(1 - \sqrt{t}/\sqrt{\tau})$.
(*a*) Standard electrolyte.
(*b*) Standard electrolyte plus 4 mgm./l. sodium chloride.
(*c*) Standard electrolyte plus 45 mgm./l. sodium chloride.
(*d*) Standard electrolyte plus 5 mgm./l. gelatin.
(*e*) Standard electrolyte plus 25 mgm./l. gelatin.
(*f*) Standard electrolyte plus 250 mgm./l. gelatin.

**Concentrations are used in place of activities for convenience.*

should be a straight line with slope $0.030/\alpha$ and intercept on the ΔE axis roughly equal to $0.030/\alpha \log(i/I_0)$. Hence approximate values of α and I_0 can be calculated.

Fig. 5 shows the plot of ΔE against $-\log(1-\sqrt{t}/\sqrt{\tau})$ for a number of experiments. Curve (*a*) was obtained by taking the average of 12 individual experiments made in the absence of addition agents, and Curve (*e*) is the average of eight experiments with 25 mgm. per liter of gelatin in the electrolyte. The other curves represent single determinations. The polarization values were often erratic in the early part of electrolysis, especially in the presence of addition agents, but after two minutes the plots were essentially straight lines. From these and similar plots the values for α and I_0 in Table I were calculated. There was some variation in I_0 values between individual experiments but the α values were relatively constant for any given set of conditions.

TABLE I

EFFECT OF ADDITION AGENTS ON α AND I_0

Expt.	Amt. of agent	α	I_0(amp./cm.^{2} × 10^3)
Absence of addition agents			
1		0.32	1.94
2		0.32	1.52
3		0.35	1.41
4		0.35	1.14
5		0.34	1.46
6		0.34	1.38
7		0.33	1.31
8		0.33	1.44
9		0.33	1.49
10		0.32	1.52
11		0.33	1.65
12		0.32	1.46
		Av. 0.33	Av. 1.48
Gelatin in the electrolyte			
	25 mgm./l.	0.20	0.92
	"	0.20	0.98
	"	0.20	0.98
	"	0.20	0.81
	"	0.20	0.69
	"	0.20	0.80
	"	0.20	0.71
	"	0.20	0.80
		Av. 0.20	Av. 0.84
	5	0.22	0.80
	10	0.21	0.71
	100	0.19	0.64
	250	0.18	0.69
Sodium chloride in the electrolyte			
	4	0.34	0.67
	45	0.34	0.52

As indicated above, Equation (2) shows that polarization may be considered as consisting of two parts: (*a*) a part dependent on t (the first term on the right-hand side of the equation), and (*b*) a part relatively independent of t (second term on the right-hand side), providing i/I_0 and ΔE are large. The results in Fig. 5 and Table I show that gelatin alone in the electrolyte affects both of these parts by decreasing both α and I_0 whereas chloride alone apparently affects the part independent of t only, by causing a decrease in I_0. The α and I_o values could not be calculated, in this way, for the experiments in which combinations of gelatin and chloride were used in the electrolyte because the plots of ΔE against $-\log(1-\sqrt{t}/\sqrt{\tau})$ were not straight lines. Possibly the condition of the surface of the cathode was continually changing during electrolysis, in which case neither α nor I_0 are necessarily constant during an experiment.

REFERENCES

1. FISCHER, H. (*a*) Kolloid Z. 106: 50. 1944.
   (*b*) Z. Electrochem. 49: 342; 376. 1943.
2. GAUVIN, W. and WINKLER, C. A. Can. J. Research, B, 21: 125. 1943.
3. GLASSTONE, S. Introduction to electrochemistry. D. Van Nostrand Co. Inc., New York. 1947.
4. HUNT, L. B. J. Phys. Chem. 36: 2259. 1932.
5. JACQUET, P. (*a*) Compt. rend. 196: 921. 1933.
   (*b*) Trans. Electrochem. Soc. 63: 83. 1933.
6. MCCONNELL, W. B., MANDELCORN, L., GAUVIN, W., and WINKLER, C. A. J. Electrochem. Soc. 99: 84. 1952.
7. PETROCELLI, J. V. J. Electrochem. Soc. 98: 187. 1951.
8. RIUS, A., LLOPIS, J., and POLO, S. Anales real soc. espan. fis. y quim., B, 45: 469. 1949.
9. SAND, H. J. S. Phil. Mag. 1 (Ser. 6): 45. 1901.
10. TAFT, R. and MESSMORE, H. E. J. Phys. Chem. 35: 2585. 1931.
11. TURNER, R. C. and WINKLER, C. A. J. Electrochem. Soc. 99: 78. 1952.
12. VETTER, K. Z. Phys. Chem. 194: 284. 1944-50.

PURIFICATION AND COMPOSITION OF A POLYURONIDE HEMICELLULOSE ISOLATED FROM WHEAT STRAW[1]

By G. A. Adams and A. E. Castagne

ABSTRACT

A homogeneous polyuronide hemicellulose has been prepared from a crude hemicellulose isolated from wheat straw holocellulose by hot water. The method of preparation consisted of fractional precipitation of the acetylated hemicellulose from chloroform solution by petroleum ether. Approximately 50% of the recovered fractions consisted of a polysaccharide made up of 28 moles of D-xylose, 5 moles of L-arabinose, and 3 moles of uronic acid and having an $[\alpha]_D^{20} = -92°$ and a molar ratio, methoxyl: uronic anhydride, of approximately 1.0. Intrinsic viscosity measurements indicated a degree of polymerization of approximately 30. Separation of hexosans present in the original hemicellulose showed that they were not chemically combined with the main pentosan fraction. They were recovered admixed with short chain pentosan material containing a higher proportion of arabinose to xylose than the main homogeneous fraction.

INTRODUCTION

Most hemicellulose preparations from the structural portions of plants appear to be mixtures of polysaccharide materials and separation into homogeneous fractions has not been convincingly achieved. Many of the fractions which are predominantly pentosan also contain hexosans in varying amounts. Investigations on various wood hemicelluloses indicate that anhydroglucose units may be chemically combined in the xylanglucuronide molecule (3, 10). In agreement with this view is the finding that glucose units are associated with xylans prepared from herbaceous plants (4, 5).

Attempts to produce homogeneous polysaccharides have been made by successive extractions of cellulosic materials with various mild solvents, and series of hemicelluloses which show systematic variations in composition and molecular size have been prepared (8, 9). In a similar investigation, the present authors isolated a hot-water soluble hemicellulose fraction from wheat straw holocellulose (1). Fractional precipitation with ethanol yielded a main component equivalent to about 10% of the original holocellulose. On acid hydrolysis, this fraction was shown to be composed of xylose (62%), arabinose (12%), glucose (5%), galactose (1%), and hexuronic acid (8%). The apparent complexity of this hemicellulose suggested that it might be a mixture of pentosans and hexosans rather than a chemical entity.

The purpose of the present investigation was to attempt a further purification of this hemicellulose of wheat straw and if possible to prepare a homogeneous component which would be suitable for structural studies.

The method involved acetylation of the material, followed by fractional precipitation from chloroform solution with petroleum ether. Isolated fractions were analyzed for component sugars, uronic acid anhydride, and methoxyl content. Intrinsic viscosities of the fractions were also determined.

[1] *Manuscript received March 10, 1952.*
Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as Paper No. 138 on the Uses of Plant Products and as N.R.C. No. 2752.

EXPERIMENTAL AND RESULTS

Preparation of the hot-water soluble hemicellulose material from wheat straw holocellulose and preliminary fractionation with ethanol have been previously described (1). The first fraction precipitated by ethanol and comprising about 85% of the entire hot-water soluble material was used in this study. Analysis showed the following composition (moisture ash-free basis): $[\alpha]_D^{25} - 67°$, pentosan 64.7%, uronic acid anhydride 8.44%, methoxyl 1.79%, and acetyl 3.17%.

Acetylation

Preliminary experiments on acetylation of the hemicellulose fractions were carried out on 0.5 gm. samples using the method of Carson and Maclay (5). Recovery of the acetates by pouring the acetylated mixture into ice and water yielded a white flocculent product which was readily dried from alcohol and ether to a white powder. While the recovered material had an acetyl content of 39.3% (calculated for pentosan diacetate 39.8%) the yield of acetylated material was low (80%). Modification of the procedure whereby the acetates were precipitated in several volumes of cold 95% ethanol gave quantitative yields although two acetylations were necessary to bring the acetyl content to 39.3%. Neither prolonged acetylation (44 hr.) nor two additional treatments increased the acetyl content beyond this value.

The following procedure was adopted for the preparation of large samples: 20 gm. of the hemicellulose (dried *in vacuo* over phosphoric anhydride) was dispersed in 400 ml. of formamide by stirring at 65°C. for one hour. The solution was then poured into 400 ml. of pyridine (freshly distilled) and the temperature of the solution reduced to 20°C. Sixty-four milliliters of acetic anhydride was added in 16 ml. lots at hourly intervals and the solution was stirred in a moisture-free atmosphere for 24 hr. The acetylated mixture was then poured slowly into six volumes of cold 95% ethanol with vigorous stirring. The gray flocculent precipitate was centrifuged off and dried with alcohol and ether. For the second acetylation the dried acetate (acetyl content 37.8%) was dissolved directly in pyridine and the first treatment repeated. The recovered acetates had an acetyl content of 39.1%.

Fractionation of Acetylated Hemicelluloses

The method consisted of fractional precipitation of the acetates from chloroform solution using petroleum ether (boiling range 65-100°C.). Acetate (25 gm.) was dissolved readily in 2500 ml. of chloroform and filtration through a 'coarse' glass filter gave a clear colorless solution. Petroleum ether was then added slowly while the chloroform solution was vigorously stirred. When the cloudy suspension which appeared began to coalesce into definite flocs on standing for 5-10 min., the solution was centrifuged and the fraction recovered. This procedure was repeated until a total of 14 fractions had been separated. Fractions 1–9 were slightly brown colored gels while the remainder were white in color and grainy in texture. All fractions were reprecipitated once from chloroform solution. The gels were repeatedly washed with petroleum ether and finally with several changes of ethyl ether. After removal of the ether and

drying of the fractions *in vacuo* over phosphoric anhydride they were recovered as dry friable solids.

Table I gives the yields, acetyl contents, and specific rotations of the acetylated fractions. The close similarity of the specific rotations of Fractions 2–9 comprising about 75% of the material indicated strongly that they were of similar chemical composition. The order of rotation (− 92°) suggested that the sugar units were linked predominantly in the β configuration. The specific rotation of fraction No. 1 was not determined owing to the difficulty of obtaining adequate solubility in chloroform. Acetyl values showed that some of the first fractions were not fully acetylated but the high values of some of the last fractions exceeded the calculated value for fully acetylated pentosans indicating the presence of hexosan material. The higher acetyl values and lower

TABLE I

YIELDS, ACETYL CONTENT, AND SPECIFIC ROTATIONS OF ACETYLATED HEMICELLULOSES FRACTIONATED FROM CHLOROFORM SOLUTION BY PETROLEUM ETHER

Fraction No.	Wt. of fraction (gm.)	Fractions as percentage of total wt.	Acetyl (%)	$[\alpha]_D^{20}$
1	0.6678	2.67	35.8	——
2	3.6777	14.70	35.8	− 96
3	2.9018	11.61	35.8	− 88
4	1.5201	6.09	35.8	− 92
5	1.4208	5.69	36.6	− 92
6	2.0834	8.35	38.4	− 92
7	1.8137	7.22	38.4	− 92
8	2.0782	8.33	39.5	− 96
9	2.3841	9.55	39.7	− 92
10	1.7632	7.06	39.7	− 80
11	1.7053	6.83	40.5	− 80
12	1.2743	5.10	40.9	− 64
13	0.7713	3.10	42.7	− 36
14	0.1172	0.47	43.5	——
Total	24.1789	96.77		

specific rotations of Fractions 10–14 supplied indirect evidence that the hexosans had been largely separated from the bulk of the pentosan material. Confirmation of this observation is supplied in a later section dealing with the identification and determination of the component sugars of the respective fractions.

Intrinsic Viscosities of Acetylated Fractions

Intrinsic viscosity measurements on the various acetylated fractions were made to determine the extent to which fractional precipitation had separated materials of approximately the same molecular size. Solutions containing 0.5% of each fraction were made up in chloroform from which 0.25%, 0.125%, and 0.0625% solutions were also prepared by dilution. Flow times were measured on freshly prepared solutions in an Ostwald-Cannon-Fenske type viscometer (ASTM 50) in a water bath maintained at 25° ± 0.02°C. Flow time measure-

ments were made in triplicate or until agreement to ± 0.1 sec. was obtained. Specific viscosity values were calculated from the expression

$$\frac{T - T_0}{T_0 C}$$

when T = flow time of solution in seconds,

T_0 = flow time of solvent in seconds,

and C = concentration of solution in grams per liter. A plot of specific viscosity against concentration of solution permitted extrapolation of the curve to zero concentration, the intercept of the curve on the ordinate giving the intrinsic viscosity $[\eta]$. All curves were straight lines for the concentration used. Estimation of the degree of polymerization was obtained by substituting the found values for $[\eta]$ in Staudinger's equation

$$[\eta] = KmP$$

where P = degree of polymerization
and $Km = 11 \times 10^{-4}$ (8).

Intrinsic viscosities and degrees of polymerization given in Table II show a progressive decrease in viscosity from Fraction 2 to 14. Insolubility of Fraction

TABLE II
INTRINSIC VISCOSITY AND DEGREE OF POLYMERIZATION OF THE HEMICELLULOSE ACETATE FRACTIONS

Fraction No.	$[\eta]$	D.P.
1	—	—
2	0.036	32
3	0.032	29
4	0.032	29
5	0.030	27
6	0.030	27
7	0.030	27
8	0.029	26
9	0.026	24
10	0.026	24
11	0.022	20
12	0.020	18
13	0.016	14
14	0.012	11

1 in chloroform prevented estimation of its viscosity. The variations in viscosity parallel the solubility of the fractions; the most insoluble fractions having the highest viscosities. With the exception of Fraction 2 which had a somewhat higher viscosity, Fractions 2–6 had very similar viscosities. These comprise the same series that had similar specific rotations. The data therefore indicated that these fractions were nearly identical in chemical composition and molecular size.

Deacetylation of Fractions

For further study, the fractions were deacetylated by the following method.

Swelling of the fractions was brought about by suspension in acetone for 12 hr. Normal potassium hydroxide in slight excess of that required to neutralize the acetyl content (as acetic acid) was added and the solution allowed to stand 48 hr. at 5°C. The solution was then titrated with 1.0 *N* hydrochloric acid to the neutral point using phenolphthalein as indicator. An excess of ethanol was added to precipitate the hemicellulose. The product was thoroughly washed with alcohol and ether and dried to constant weight *in vacuo* over phosphoric anhydride. The deacetylated hemicelluloses were recovered in quantitative yield.

Analysis of Hemicellulose Fractions

Methoxyl and Uronic Acid Anhydride

All fractions were analyzed for methoxyl and uronic acid anhydride content by methods previously described (2). The results (Table III) show that the

TABLE III
METHOXYL AND URONIC ACID ANHYDRIDE CONTENT OF HEMICELLULOSE FRACTIONS

Fraction No.	Methoxyl (%)	Uronic acid anhydride (%)	Molar ratio, methoxyl/uronic anhydride
1	1.75	10.88	0.9
2	2.17	10.60	1.1
3	1.81	9.40	1.1
4	1.85	10.28	1.0
5	1.85	9.24	1.1
6	1.66	8.44	1.1
7	1.63	8.80	1.0
8	1.66	9.24	1.1
9	1.46	7.36	1.1
10	1.38	6.72	1.1
11	1.21	6.60	0.9
12	1.29	8.76	0.8
13	1.65	9.08	1.0

hemicelluloses from the most insoluble acetylated fractions were highest in uronic acid content. There was a progressive drop in uronic acid content in the more soluble fractions with the exception of No. 12 and 13; no satisfactory explanation has been found for the increase in the latter. The methoxyl–uronic acid molar ratio was approximately unity for all fractions, thus indicating that each uronic acid residue contained one methoxyl group.

Sugar Components

All hemicellulose fractions were hydrolyzed by heating 100 mgm. in 100 ml. of 1% sulphuric acid (*v*/*v*) under reflux in a boiling water bath. The hydrolysis was continued until the reducing power of the solution became constant. The solution was brought to pH 6.5 with barium carbonate, the barium sulphate precipitate filtered off and thoroughly washed with hot water. The filtrate and washings were concentrated under reduced pressure, clarified with charcoal, and brought to a volume of 25 ml. Component sugars of each hydrolyzate were detected by a descending paper chromatography technique (11).

Chromatograms were run for approximately 16 hr. in a sealed glass jar using a solvent system of ethyl acetate–water–pyridine (2:2:1). The developing indicator was aniline phthalate and the reference sugars were D-xylose, L-arabinose, D-glucose, and D-galactose. Fractions 1–6 inclusive contained only xylose and arabinose; Fractions 7 and 8 contained in addition traces of glucose and galactose; Fractions 9–14 contained xylose, arabinose, glucose, and galactose. These results confirmed earlier conclusions that Fractions 1–6 contained only pentosan material and that separation of the hexosan from the bulk of the pentosan had been achieved. Separation of pentosan and hexosan material by fractional precipitation showed that these components were not linked chemically.

Fractions 2, 5, 7, 9, 11, and 13 were selected for quantitative determination of sugar components by methods previously described (1). The results are given in Table IV. Fractions 2 and 5 contained about 70% xylose and 13.5% arabinose. Fractions 7 and 9 contained approximately the same amount of pentoses and, in addition, small amounts of glucose and galactose. In Fractions 11 and 13 the ratio of xylose to arabinose decreased, the total pentose sugar content decreasing while the hexose sugar increased. The remainder of the material in the various hydrolyzates was presumably an aldobiuronic acid complex containing a hexuronic acid and xylose as previously found in the original unfractionated hemicellulose (1). The close similarity of the xylose, arabinose, and uronic acid content of Fractions 2 and 5 indicates that Fractions 1–6 contained a polyuronide hemicellulose of substantially homogeneous chemical composition. This material constituted about 50% of the original hot-water soluble hemicellulose. Apparently Fractions 7, 8, and 9 contain the same homogeneous pentosan fraction admixed with a small amount of the hexosan fraction. The lower xylose to arabinose ratio in Fractions 11 and 13 indicated pentosans of different composition. Complete separation of the pentosan and hexosan in these fractions by the present method appears unlikely because of the similar solubilities of the short chain acetates.

TABLE IV

SUGAR COMPONENTS OF HEMICELLULOSE FRACTIONS, %

Fraction No.	Xylose	Arabinose	Glucose	Galactose
2	69.8	13.6	nil	nil
5	73.0	13.7	nil	nil
7	68.7	12.2	3.8	2.4
9	70.6	13.9	3.5	2.7
11	59.2	16.1	7.9	7.3
13	41.3	19.6	13.7	11.4

DISCUSSION

In the present investigation, a pentosan fraction of wheat straw hemicellulose was isolated free of hexosans. About 50% of the fractionated material was hexosan free and a further 25% contained only a small amount of hexose sugar. The ratio of xylose to arabinose (5.2:1) and the uronic acid content of about 10% indicated a polyuronide hemicellulose unit composed of 28 moles

xylose, 5 moles arabinose, and 3 moles uronic acid as the main homogeneous component.

Studies on English oak (10) and lemon and black locust (3) indicated that the hemicellulose fractions contained anhydroglucose units in chemical combination. Evidence was based on the fact that enzymatic hydrolysis removed hexosan more slowly than was to be expected from a physical mixture of xylan and starch. None of the preparations were subjected to extensive purification procedures and the possibility that the glucose may have originated from wood starches seems reasonable. Other reports of glucose as a constituent of wood hemicellulose (9, 12) have not been substantiated by the actual identification of the sugar in the isolated fractions. While glucose may be present as a component of crude hemicellulose from a wide variety of plant sources, its association as an integral part of the hemicellulose molecule is subject to doubt until more rigorous purification procedures are applied.

Although, in the present study, the constancy of the xylose to arabinose ratio is strongly indicative of chemical union, the possibility of a mixture of xylan and araban cannot be excluded. Chanda *et al.* (6) have recently isolated an undegraded xylan of esparto grass from which arabinose had been completely removed by treatment with alkaline copper hydroxide. Electrophoretic studies by Isherwood (7) indicated that complex polysaccharides may be mixtures rather than chemical compounds. On the other hand, Boggs *et al.* (4) have reported arabinose as a component of the xylan of wheat straw—a finding in agreement with that reported here.

The heterogeneity of the hemicelluloses which are present in the cell wall is well known. Pentosans of cellulosan origin contain almost exclusively xylose and a small amount of uronic acid in contrast to material of polyuronide origin which contains greater amounts of arabinose and uronic acid in proportion to the xylose content. Pentosans prepared by extraction of plant material with strong alkali would be expected to contain a high proportion of the cellulosan fraction and to differ from polyuronide material prepared by extraction with mild reagents. With such a graded series of hemicelluloses present, it is not surprising to find polyuronide fractions which vary severalfold in their xylose: arabinose ratio.

A study of the structure of the homogeneous hemicellulose material isolated in the present investigation is in progress.

REFERENCES

1. ADAMS, G. A. and CASTAGNE, A. E. Can. J. Chem. 29: 109. 1951.
2. ADAMS, G. A. and CASTAGNE, A. E. Can. J. Research, B, 27: 424. 1949.
3. ANDERSON, E., SEELEY, M., STEWART, W. T., REED, J. C., and WESTERBEKE, D. J. Biol. Chem. 135: 189. 1940.
4. BOGGS, L., CUENDET, W. S., EHRENTHAL, I., KOCH, R., and SMITH, F. Nature, 166: 520. 1950.
5. CARSON, J. F. and MACLAY, W. D. J. Am. Chem. Soc. 70: 293. 1948.
6. CHANDA, S. K., HIRST, E. L., JONES, J. K. N., and PERCIVAL, E. G. V. J. Chem. Soc. 1289. 1950.
7. ISHERWOOD, F. A. Abstract No. 339/11. Communication to 1st International Congress of Biochemistry. Cambridge, England, Aug. 14-25, 1949.
8. MILLET, M. A. and STAMM, A. T. J. Phys. & Colloid Chem. 51: 134. 1947.
9. MITCHELL, R. L., and RITTER, G. J. J. Am. Chem. Soc. 62: 1958. 1940.
10. O'DWYER, M. Biochem. J. 28: 2116. 1934; 31: 254. 1937.
11. PARTRIDGE, S. M. Nature, 158: 270. 1946.
12. THOMAS, B. B. Paper Ind. and Paper World, 27: 347. 1945.

THE ESTIMATION OF FREE AMINO ACIDS USING A MICRODIFFUSION TECHNIQUE[1]

By W. B. McConnell

ABSTRACT

The carbon dioxide evolved when α-amino acids were heated for one hour at 85°C. with ninhydrin was determined in a partially evacuated microdiffusion cell. Distillation of solvent from one chamber to the other was minimized by keeping the ionic concentrations of the reaction mixture and absorbing mixture approximately equal. The method was useful for samples of amino acids which liberated from 0.06 to 0.3 mgm. of carbon dioxide. The average deviation from the mean was somewhat less than 1% for samples liberating 0.2 mgm. of carbon dioxide. Use of the method for routine analysis of enzymatic digests resulted in substantial saving of time and material.

INTRODUCTION

Van Slyke, Dillon, MacFadyen, and Hamilton (7) have shown that the reaction of ninhydrin (triketohydrindene hydrate) with α-amino acids (6) in acid solution at 100°C. liberates a stoichometric amount of carbon dioxide. The reaction was found to be highly specific for α-amino acids (7) and provided a method for following protein degradation by hydrolytic agents such as acids and enzymes.

The object of this paper is to describe a method in which the techniques of microdiffusion (2) are used to substantially shorten and simplify the use of the ninhydrin reaction for determining free amino acids. Partial evacuation of the microdiffusion cells after the addition of the reagents permitted the storing of the cells at elevated temperatures during the reaction in order to accelerate the liberation of carbon dioxide. The heating of a sample with ninhydrin and the collection of the evolved carbon dioxide were therefore done in a single operation.

EXPERIMENTAL

Apparatus

Microdiffusion cells.—Pyrex glass cells as shown in Fig. 1 were used. The diameters of the chambers were similar to those of the standard Conway unit No. 2 (2). To provide for evacuation of the cell the top consisted of a ground glass joint fitted with a stopcock.

Evacuation chamber.—A 9 liter bottle, fitted with a stopcock and a rubber connecting hose, was used to rapidly adjust the pressure in the cells. The air pressure in the chamber was maintained at a value of 21 cm. of mercury less than atmospheric pressure. About 20 evacuations could be done before readjustment of the pressure was necessary.

Microburette.—Details of the construction of a suitable microburette are given by Neish (5). The volume of liquid delivered as a result of turning a micrometer screw through one division was 0.000604 ml.

[1] *Manuscript received February 8, 1952.*
Contribution from the National Research Council, Prairie Regional Laboratory, Saskatoon, Sask. Issued as Paper No. 137 on the Uses of Plant Products and as N.R.C. No. 2747. Presented in part at the 34th Annual Conference, The Chemical Institute of Canada, Winnipeg, June 18-20, 1951.

Magnetic stirring assembly.—Small magnetic stirrers suitable for agitating the solution in the inner chamber during a titration were made by sealing a short length of a small nail in glass tubing.

Pipettes.—A 0.2 ml. Chaney pipette (1), constructed as described by Neish (5), was used to deliver alkali to the inner chamber. A rubber bulb automatic pipette* was convenient for rapid addition of ninhydrin solution to the sample.

Air oven.—Thermostatically controlled at 85°C.±1°C. Fig. 2 shows an assembly of all the necessary apparatus except the oven.

Reagents

Ninhydrin–buffer solution.—2.5 gm. ninhydrin and 2.5 gm. citrate buffer at pH 2.5 dissolved in 100 ml. of carbonate free water. The citrate buffer was made by grinding together 10 gm. trisodium citrate ($Na_3C_6H_5O_7.2H_2O$) and 93.0 gm. of citric acid ($C_6H_8O_7.H_2O$).

Absorbing solution.—0.1 *N* solution of carbonate free sodium hydroxide containing 10 ml. of 0.1% alcoholic thymolphthalein in 100 ml. of solution. The solution was made 0.25 *N* with respect to sodium chloride.

Standard hydrochloric acid.—0.1 *N*.

Barium chloride.—0.20 *N*.

Procedure

The sample, 0.2 ml., containing from 0.02 to 0.10 mgm. of free amino acid nitrogen was pipetted into the outer chamber of the cell. Absorbing solution (0.2 ml.) was measured into the inner chamber and approximately 0.8 ml. of ninhydrin buffer solution was added to the sample in the outer chamber. The cover of the cell, which had been liberally coated with a temperature resistant grease,† was put into position and the cell connected briefly to the evacuation chamber. The cell was rotated gently to mix the reactants and placed in the oven for one hour at 85°C. The liberated carbon dioxide was absorbed by the alkali in the inner chamber. The sodium chloride in the absorbing solution tended to equalize the ionic concentrations of the solutions and so reduced the tendency for liquid to distill from one chamber to the other. After the cells had cooled for 30 min. they were opened and three drops of barium chloride were added to the absorbing solution to precipitate the carbonate. The excess alkali was then determined by titration with standard hydrochloric acid. Magnetic stirring was employed during the titration.

The amount of free amino acid in the sample was calculated by taking the difference in the volume of standard acid used for the titration from the volume that was used for a blank analysis of the reagents. Theoretically a mole of amino acid liberates two equivalents of carbon dioxide. The amount of carbon dioxide obtained for the blank analysis was usually very low. In the present work the results are expressed in terms of the amount of carbon dioxide collected.

* *Pipettes of this type are sold by Alfred Becknell Associates, 243 Broadway, Cambridge, Mass.*

† *A silicone grease sold by Dow Corning was found most satisfactory. If after several determinations the cells became contaminated with the grease they were cleaned by immersion in alcoholic potassium hydroxide for 15 min.*

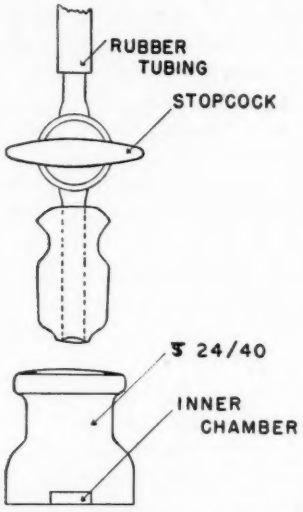


FIG. 1. Microdiffusion cell for use with reduced pressure.

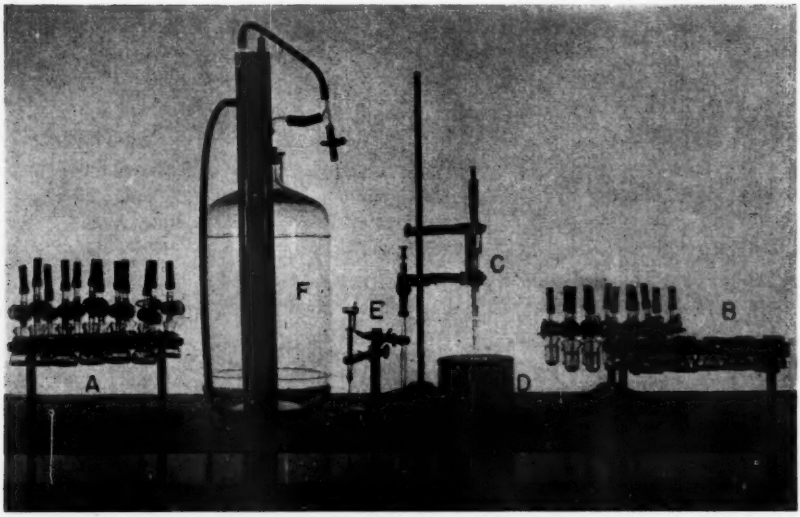


FIG. 2. Assembly of apparatus for amino acid assay.
A. Assembled microdiffusion cells.
B. Dismantled cells.
C. Microburette.
D. Magnetic stirrer.
E. Pipettes.
F. Evacuation chamber.

If the sample to be analyzed contains an appreciable amount of carbonate it should be removed by boiling the acidified sample. A satisfactory correction for small amounts of carbonate can be obtained from a determination done in the absence of ninhydrin. Acetates also interfere seriously with the determination, because, for the conditions described, some acetic acid diffuses into the center chamber. Chlorides in appreciable concentrations also interfere. (Almost 3% of the acid in a blank sample consisting of 0.05 *N* hydrochloric acid passed into the absorbing solution.)

The actual working time, exclusive of time spent in cleaning and arranging the equipment, was from three to four minutes per determination.

EXPERIMENTAL RESULTS AND DISCUSSION

The method was applied to solutions of pure amino acids of known concentration. Reagent grade, twice recrystallized materials were used to prepare a series of solutions with amino acid concentrations ranging from about 0.006 *N* to about 0.035 *N*. The results given in Fig. 3 show that the amount of carbon dioxide collected increases linearly with the amount of amino acid in the sample. The yields of carbon dioxide expressed as per cents of theoretical were calculated from the slopes of the lines. They are as follows: glycine 94%, leucine 100%, alanine 102%, glutamic acid 101%. The deviation from the

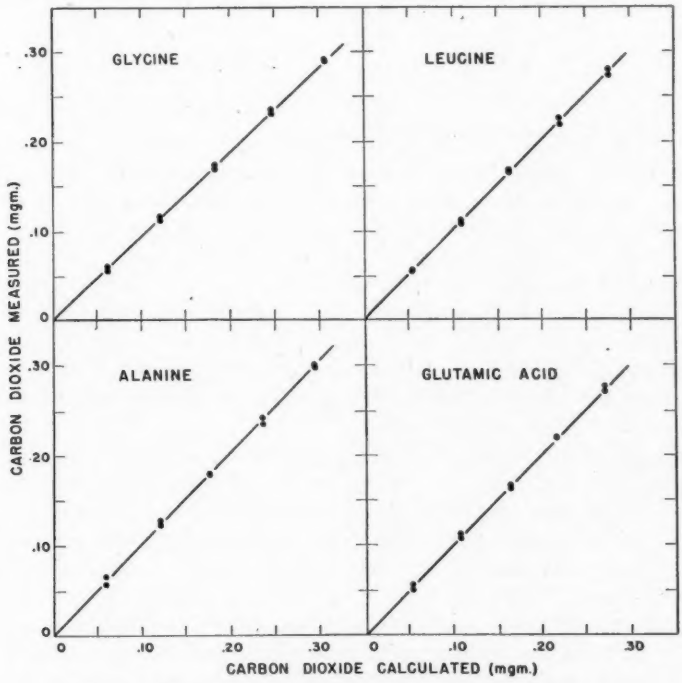


FIG. 3. The yield of carbon dioxide from some amino acids.

mean for all pairs of duplicate measurements in the data used for Fig. 3 is equivalent to about 0.0018 mgm. of carbon dioxide. This is somewhat less than 1% for a sample liberating 0.2 mgm. of carbon dioxide.

Table I provides a comparison of amounts of carbon dioxide obtained by the present method from pure amino acids with those obtained by Van Slyke *et al.* by their gasometric method in which the reactants, buffered at pH 2.5, were heated for seven minutes in a boiling water bath. The first five amino acids listed in the table represent those that were previously found to give theoretical yields. For these the results obtained by the microdiffusion technique were also close to theoretical. Glycine and tryptophane give low yields

TABLE I

QUANTITY OF CARBON DIOXIDE LIBERATED BY AMINO ACIDS

Amino acid	Moles CO_2 evolved/mole amino acid	
	Microdiffusion method	Gasometric method**
Leucine	1.00	1.00
Alanine	1.02	1.00
Glutamic acid	1.01	1.00
Arginine	1.00	1.00
Valine	1.02	1.00
Glycine	0.94	0.95
Tryptophane	0.96	0.90
Aspartic acid	1.88	2.00
Cystine	2.16*	1.89

* *pH — 1.6.*
** *Values reported by Van Slyke and co-workers.*

by both methods. Results of the gasometric method indicate that both carboxyl groups of aspartic acid react quantitatively while in the present work only about 1.88 moles of carbon dioxide were obtained per mole of the acid. A mole of cystine should yield two moles of carbon dioxide but at a pH of 2.5 values somewhat less than this were reported for the gasometric procedure. In the present work a yield considerably in excess of the theoretical two moles was obtained. The excess sulphuric acid used to dissolve the cystine was found to have reduced the pH of the reaction mixture to 1.6. The high yield of carbon dioxide from cystine at the lower pH is in accord with some observations Van Slyke made with more strongly acid solutions (7).

Almost theoretical yields of carbon dioxide can be obtained from glycine by prolonging the reaction time, as is shown by the following results obtained with variable heating time: one and one-half hours, 95.5%; two hours, 97.8%; and two and one-half hours, 99.5%. The amounts of carbon dioxide evolved from alanine, leucine, and glutamic acid are not appreciably increased by extending the heating time beyond one hour. Experiments done with various temperatures indicated that 85°C. gave a suitable reaction rate. Although it appeared that, with care, the technique could be used to at least as high as 95°C., the lower temperature was more convenient and was used for all subsequent analyses. No measurable differences in results were observed when the cooling

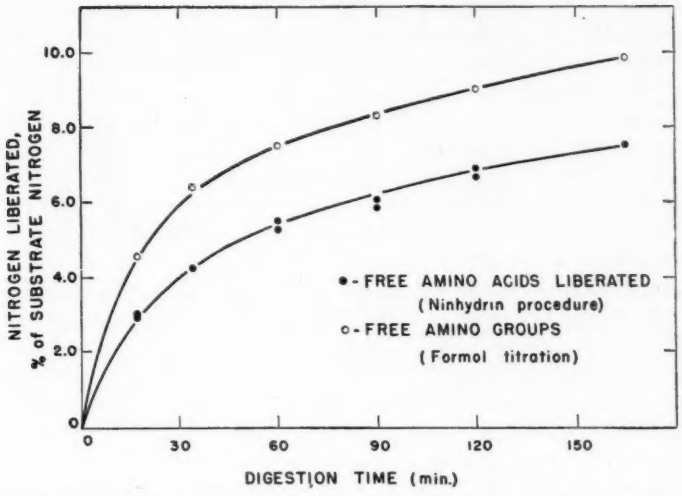


FIG. 4. The digestion of gelatin with trypsin. Original gelatin concentration 2½%; enzyme concentration 0.04% of 1:300 commercial trypsin preparation.

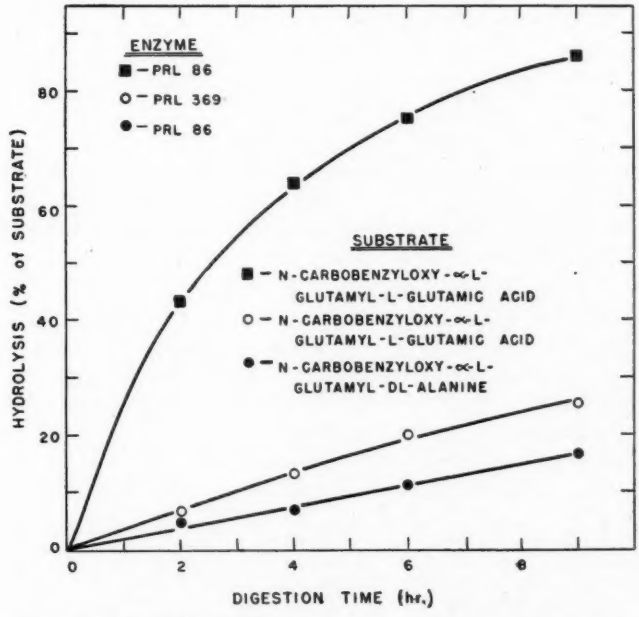


FIG. 5. The hydrolysis of some synthetic substrates with mold proteases.

time was extended beyond one-half hour. The results reported in this work were obtained with a pH of 2.5, because it has been suggested as the most satisfactory for measuring the free amino acid content of protein hydrolyzates (7).

The method was used to follow enzymatic release of amino acids from proteins. In the example given (Fig. 4) a 2.5% solution of gelatin was incubated at 37°C. with a 0.04% solution of trypsin (1:300 obtained from Baltimore Biological Laboratory Inc.). The digestion mixture was buffered at pH 7. The figure also contains hydrolysis data as obtained by a formol titration procedure (4).

Fig. 5 shows some preliminary results that were obtained when the method was used to follow the hydrolysis of some synthetic substrates by crude mold proteases. The enzymes were supplied by W. M. Dion who prepared them according to methods previously described (3). Calculation of the extent of hydrolysis is based on the fact that hydrolysis of each mole of the dipeptide derivative results in the liberation of one mole of a free amino acid. The method is attractive for work of this kind because small samples are required, and because the necessity for an acid–base titration directly on a buffered digestion mixture is avoided.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance of Mr. J. A. Trew who prepared the synthetic substrates used in this work and who participated in the hydrolysis measurements on these materials.

REFERENCES

1. CHANEY, A. L. Ind. Eng. Chem. Anal. Ed. 10: 326. 1938.
2. CONWAY, E. J. Microdiffusion analysis and volumetric error. Revised Ed. Crosby Lockwood and Son, London. 1947.
3. DION, W. M. Can. J. Research, C, 28: 586. 1950.
4. MCCONNELL, W. B. Can. J. Research, C, 28: 600. 1950.
5. NEISH, A. C. Analytical methods for bacterial fermentations. National Research Council of Canada Report 46-8-3 (Revised), Saskatoon, Sask. 1950.
6. RUHEMANN, S. J. Chem. Soc. (Trans.) 99 :792; 1306; 1486. 1911.
7. VAN SLYKE, D. D., DILLON, R. T., MACFADYEN, D. A., and HAMILTON, P. J. Biol. Chem. 141: 627. 1941.

THE DECARBOXYLATION OF ANTHRANILIC ACID[1]

By W. H. Stevens, J. M. Pepper,[2] and M. Lounsbury

ABSTRACT

Anthranilic acid is known to decarboxylate on being heated above its melting point, or on being boiled in water. We have found that the aqueous decomposition can be acid catalyzed, but that, after the concentration of added mineral acid approximates that of the anthranilic acid, the reaction rate decreases with increasing mineral acid concentration. A mass spectrometer study of the carbon dioxide produced in the decomposition has shown that C^{12}-carboxyl anthranilic acid decomposes at the same rate as C^{13}-carboxyl anthranilic acid. Thus, unlike all other organic acid decarboxylations in which an "isotope effect" has been searched for thus far, the decarboxylation of anthranilic acid does not show an "isotope effect". From the experimental facts available, it appears that the mechanism of the decarboxylation is best explained as bimolecular electrophilic substitution, with the attack of a proton being the rate determining step. While other possibilities are not entirely excluded, a proton attack on the α carbon of the zwitterion is the detailed mechanism suggested as being most probable.

INTRODUCTION

The effect on the chemical reactivity of a molecule caused by the substitution of a lighter or heavier isotopic atom for some atom in that molecule is now referred to in the literature simply as an "isotope effect". Recently in this laboratory, we were looking for a decarboxylation reaction in which to study the relative magnitudes of the isotope effects of C^{14} and C^{13}. The results of such a study using mesitoic acid have been communicated briefly in a letter (11) to the Journal of Chemical Physics and will be reported more fully later. The first reaction investigated, however, was the decarboxylation of anthranilic acid. In preliminary experiments with ordinary, unlabeled anthranilic acid, it was found that the rates at which C^{13}-carboxyl and C^{12}-carboxyl anthranilic acids decarboxylate are experimentally identical. Since a C^{13} isotope effect of 3 to 4% has been found in the decarboxylation of such different monobasic acids as mesitoic (3) and trichloroacetic (1), the anthranilic acid result is of interest and proves to be a source of information for elucidating the mechanism of this particular decarboxylation.

Anthranilic acid decarboxylates when heated above its melting point (8). It also decarboxylates in boiling water and the decomposition has been reported to be first order (7). We have looked for an isotope effect in both the thermal and aqueous decarboxylations and, in addition, in an "acid catalyzed" aqueous decarboxylation. This paper reports the results obtained and proposes a mechanism for the decarboxylation to account for the known experimental facts and, in particular, for the absence of an isotope effect.

[1] *Manuscript received February 11, 1952.*

This paper is based on a portion of a thesis to be presented by W. H. Stevens in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Graduate School of Hamilton College, McMaster University, Hamilton, Ont. The work was carried out in the Chemistry Branch, Atomic Energy Project, National Research Council, Chalk River, Ont. Issued as N.R.C. No. 2742.

[2] *Associate Professor of Chemistry, University of Saskatchewan, Saskatoon, Sask.*

EXPERIMENTAL

Eastman Kodak Co. anthranilic acid was recrystallized twice from hot water, with animal charcoal being used in the first recrystallization. The product was vacuum dried (m.p.144-145°C.) and stored in a desiccator over silica gel.

Thermal Decarboxylation

Pawlewski (8) found that anthranilic acid decarboxylated on being heated above its melting point to give aniline and carbon dioxide.

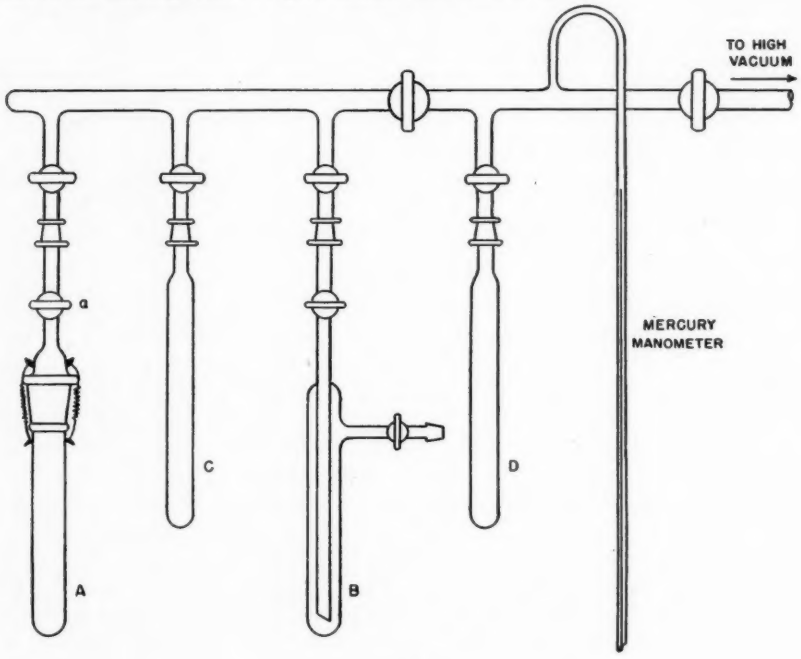


FIG. 1.

Since anthranilic acid sublimes very readily, and since the carbon dioxide produced in the decarboxylation was required for mass spectrometer analysis, the method adopted for thermal decomposition was as follows: In a typical run, 50 to 150 mgm. of acid was placed in Tube *A*, approximately 40 ml. in volume. The tube was then attached to a high-vacuum line as shown in Fig. 1. Stopcock *a* was provided with a pressure fitting and the ground joint was fitted with springs. (Dow-Corning silicone high-vacuum grease was used in the stopcock and the ground joint, since the apparatus was to be heated in an oven.) The tube was evacuated to a pressure of 10^{-5} cm. of Hg, Stopcock *a* was closed, and the assembly was then removed from the vacuum line and placed for a predetermined time in an oven regulated at a desired temperature. After removal from the oven and cooling, the assembly was returned to the vacuum line and the connection above the stopcock *a* evacuated. Then, with a cellosolve–dry

ice bath around *A* to retain the aniline produced, the carbon dioxide was transferred to Tube *C* by cooling the latter in liquid nitrogen. Further purification of the carbon dioxide was effected by distilling it from *C* at cellosolve–dry ice temperature into either *B* or *D* at liquid nitrogen temperature.

In the experiments in which time and temperature conditions for approximately 10% decomposition and for complete decomposition were being determined, the carbon dioxide was frozen in *B*. This tube was then transferred to another apparatus where the carbon dioxide was slowly swept through a semimicro combustion type absorption tube filled with ascarite, using carbon dioxide-free hydrogen as sweep gas.

In the experiments where the carbon dioxide was desired for mass spectrometer analysis, it was frozen in Tube *D*. This tube and a section of the line connecting it to a capillary bore manometer were previously calibrated so that pressures could be converted into milligrams of carbon dioxide. The carbon dioxide was allowed to warm to room temperature and the temperature and manometer reading were noted. The carbon dioxide was then frozen in an evacuated 50 ml. flask containing 5 ml. of frozen, degassed, carbon dioxide-free distilled water. The flask was isolated from the vacuum line and the carbon dioxide and water were allowed to equilibrate overnight at room temperature to ensure that all samples of carbon dioxide had the same oxygen isotopic content. The carbon dioxide was then distilled between cellosolve–dry ice and liquid nitrogen temperatures, dried over phosphorus pentoxide, and then condensed into sample tubes for mass spectrometer analysis.

Aqueous Decarboxylation

McMaster and Shriner (7) found that anthranilic acid decarboxylated in boiling water. Under the conditions of their experiments, in which a solution of the acid was allowed to boil in an open flask, the decomposition was found to be first order by these authors.

The apparatus used to investigate the kinetics of the aqueous decarboxylation under the conditions used here is shown schematically in Fig. 2. A 1 gm. sample of anthranilic acid was placed in the flask and 70 ml. of carbon dioxide-free water were added. The system was swept with hydrogen until carbon dioxide-free. The flask and contents were then heated to boiling and kept at a gentle boil while a slow stream of hydrogen swept out the carbon dioxide produced. The sulphuric acid trap removed any aniline which passed the condenser, and the acid trap and calcium chloride U-tube removed water. The absorption tubes were weighed alternately after various time intervals to determine the carbon dioxide produced.

For the runs in which the carbon dioxide was required for mass spectrometer analysis, the absorption tubes were replaced by Tube *B*, shown in Fig. 1, which was surrounded by liquid nitrogen. Tube *B*, thus cooled, was shown to be efficient for condensing small quantities of carbon dioxide from a hydrogen stream by slowly generating known quantities of carbon dioxide in the line at the flask position and measuring the amount collected in Tube *B* either in the

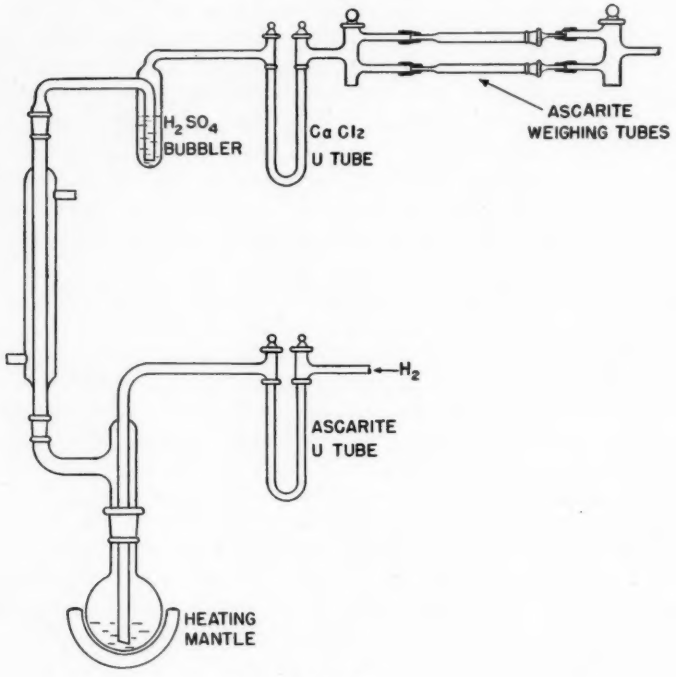


FIG. 2.

calibrated volume of the vacuum line or by absorption in ascarite. The carbon dioxide samples collected from the runs for mass spectrometer analyses were measured in the calibrated volume of the vacuum line and then treated in the same way as samples obtained from the thermal decarboxylations. Only samples of carbon dioxide from partial decarboxylations were required and obtained from the aqueous runs for mass spectrometer analyses.

Acid-catalyzed Aqueous Decarboxylation

It was found that the decarboxylation of anthranilic acid was catalyzed by the addition of small amounts of mineral acid. The kinetics of this reaction were studied over a range of mineral acid concentration and a sample of carbon dioxide produced during partial decarboxylation of anthranilic acid in sulphuric acid was analyzed with the mass spectrometer. The methods used were the same as those described for the aqueous experiments except that various concentrations of sulphuric acid from $0.25N$ to $3N$ were used instead of distilled water.

Sodium Anthranilate

An equivalent amount of $0.1N$ sodium hydroxide solution was added to a 1 gm. sample of anthranilic acid, and the volume was made up to 70 ml. with distilled water. This solution was placed in the flask used for the other aqueous

runs. The apparatus was modified by the addition of a ground-glass Y connection between the condenser and the flask. A separatory funnel was placed in one arm of the Y connection and the condenser in the other arm. After two hours of boiling, the solution was cooled and dilute sulphuric acid was added through the separatory funnel. Sufficient time was allowed for any carbon dioxide produced to be swept through the system and the ascarite tube was then weighed. No increase in weight was found.

Mass Spectrometry

The carbon dioxide samples were analyzed in a Nier-type, 90°-deflection mass spectrometer similar to one previously described (5). Mass spectra were obtained by magnetic scanning and were recorded with a Leeds and Northrup Type G Speedomax. Each analysis consisted of 6 to 10 pairs of spectrograms, a pair of spectrograms being obtained by scanning from mass 44 to 46 and then in the reverse direction from 46 to 44. Since the resolution of the mass spectrometer is one mass unit in 200, the ion peaks were completely resolved and no corrections had to be made for incomplete resolution. Systematic errors arising from fractionation of the gas sample in flowing through the spectrometer, "voltage effect" in the source, space-charge effect, secondary electron emission at the collector, and nonlinearity of the amplifying and recording systems were not present or were reduced to negligible proportions. There were no residual foreign ions in the spectrometer tube in the carbon dioxide mass range, and the almost complete absence of masses other than those arising from carbon dioxide in the spectograms was evidence of the purity of the samples. (The samples contained less than 0.1% of air.)

Absolute isotopic abundance ratios as determined by the mass spectrometer are found to vary over a period of time. Relative isotopic abundances can be determined, however, with a precision of $\pm$ 0.1% by comparing each sample with a standard. In this work, tank carbon dioxide, purified by repeated distillation, was used as a standard and each of the carbon dioxide samples was compared with it.

The C^{13}/C^{12} ratio may be obtained from the mass spectrometer ratio of mass 45 to mass 44 by correcting for the contribution of $C^{12}O^{16}O^{17}$ to the mass 45 peak as follows:

$$\frac{(45)}{(44)} = \frac{C^{13}O^{16}O^{16} + C^{12}O^{16}O^{17}}{C^{12}O^{16}O^{16}} = \frac{C^{13}}{C^{12}} + \frac{O^{16}O^{17}}{O^{16}O^{16}}$$

$$\frac{C^{13}}{C^{12}} = \frac{45}{44} - \frac{O^{16}O^{17}}{O^{16}O^{16}}$$

Since all of the carbon dioxide samples were equilibrated with water from the same source, the ratio $O^{16}O^{17}/O^{16}O^{16}$ was the same for all samples, and was determined to be 0.000802.

RESULTS

Thermal Decarboxylation

Some information on the rate of decarboxylation at 200°C. and 155°C. was obtained and is shown in Table I. This data agrees reasonably well with that

of Pawlewski (8). Although the values lack precision, they indicate that the reaction is first order as shown in Fig. 3, where log of per cent anthranilic acid remaining is plotted against time. Complete decarboxylation was never obtained at the temperatures studied. Decarboxylation does not appear to occur at temperatures below the melting point of the acid as is seen from Run 17.

TABLE I
THERMAL DECARBOXYLATION OF ANTHRANILIC ACID

Run	Temperature, °C.	Time, min.	% Decomposition
17	140 ± 4	90	0
32		20	7.0
33	156 ± 4	25	10.0
31		30	12.3
30		60	29.9
26		10	10.8
22		10	13.0
14		15	41.5
12		45	82.2
7	197 ± 3	60	89.5
8		60	92.5
9		120	96.5
10		120	94.5
11		150	95.5
Pawlewski	150	60	23.9
"	160	60	47.7
"	190	60	88.3
"	200	60	96.2

Aqueous Decarboxylation

The results of the runs to check the kinetics of the aqueous decarboxylation are given in Table II. A plot of log per cent anthranilic acid remaining against time, given in Fig. 4, shows that the reaction follows a first order rate at first, but that the rate then falls off. In view of the results of the acid catalyzed and base inhibited runs done later, it is obvious that the build-up of aniline in the reaction flask inhibits the decarboxylation. Aniline build-up probably accounts for the incompleteness of thermal decarboxylation also. The rate constant, calculated from the straight line portion of the curve, is close to that reported by McMaster and Shriner (7).

Aqueous Acid-catalyzed Decarboxylation

The results of the acid catalyzed decarboxylations are given in Table III. The reaction, in each case, is first order as far as the decarboxylation was carried. The results for $1N$, $2N$, and $3N$ sulphuric acid solutions are plotted in Fig. 4.

Mass Spectrometer Analyses

The relative abundance of C^{12} and C^{13} in the carboxyl group of the anthranilic acid used was determined from mass spectrometer analyses of the carbon dioxide obtained in thermal decarboxylations which were 96 and 98%

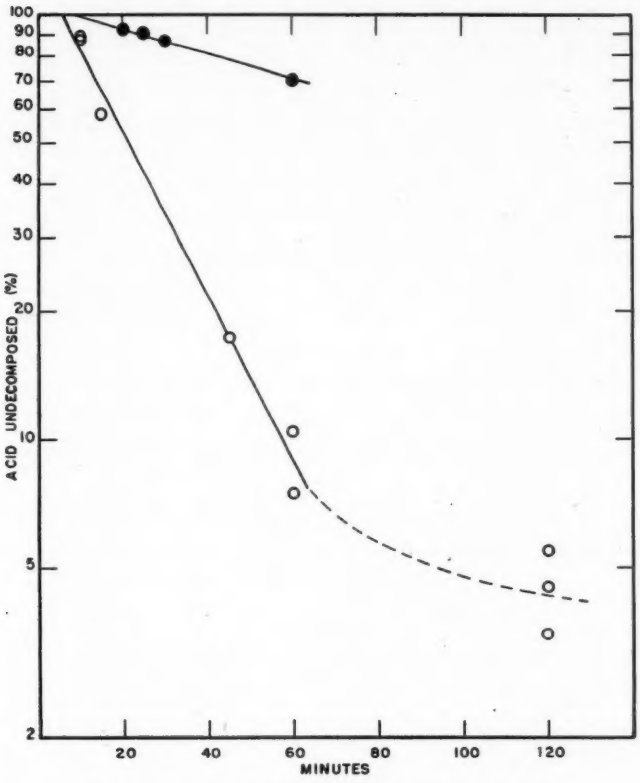


FIG. 3. Thermal decarboxylation of anthranilic acid.
● 156 ± 4°C.
○ 197 ± 4°C.

TABLE II

AQUEOUS DECARBOXYLATION OF ANTHRANILIC ACID
(~100°C.)

Time, hr.	% Decomposition	Rate constant, hr.$^{-1}$	
		Present work	McMaster and Shriner
2	5.2	0.0268	0.029
3	7.3	0.0257	0.025
4	9.6	0.0250	0.031
7	15.4	0.0240	0.025
12	18.1	0.0165	
15	22.7	0.0172	

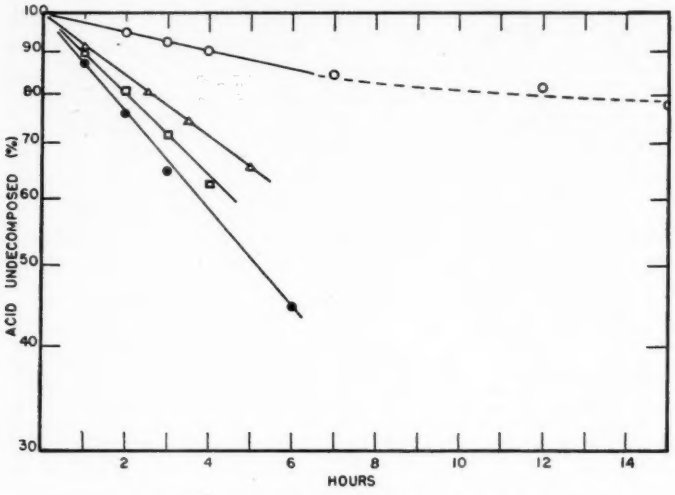


FIG. 4. Aqueous decarboxylation of anthranilic acid.
○ No mineral acid added.
● 1*N* sulphuric acid.
□ 2*N* sulphuric acid.
△ 3*N* sulphuric acid.

TABLE III

ACID CATALYZED AQUEOUS DECARBOXYLATION OF ANTHRANILIC ACID
(~100°C.)

Sulphuric acid normality	1 hr.	2 hr.	2.5 hr.	3 hr.	3.5 hr.	4 hr.	4.5 hr.	5 hr.	6 hr.	Rate constant, $hr.^{-1} \times 10^2$
0.25	12.32	22.62			37.34		45.76		55.13	13.5
0.50	12.21	23.42								13.7
0.75	13.81	25.41		35.05		43.03		49.85	56.46	14.1
1.0	12.93	24.27		35.10					55.10	13.9
2.0	10.15	19.62		28.63		37.63				11.6
3.0	8.45		19.82		25.80			34.53		8.5

TABLE IV

MASS SPECTROMETER ANALYSES

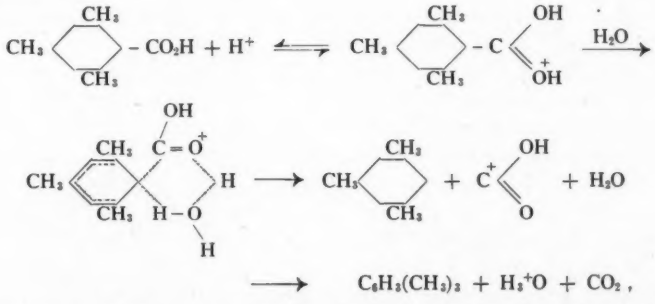
Sample No.	Decarboxylation method	% Decarboxylation	Ratio C^{13}/C^{12} in $CO_2 \times 10^4$
1*e*	Thermal 200°C.	96	108.54
3*e*	Thermal 200°C.	98	108.49
5*e*	Thermal 160°C.	67	108.51
2*e*	Thermal 160°C.	13	108.22
6*e*	Thermal 160°C.	10	108.10
7*e*	Aqueous	10	108.10
8*e*	Aqueous acid-catalyzed (1*N* sulphuric acid)	12	108.02
Tank CO_2			112.73

complete. If an isotope effect were present, this value would leave some doubt as to the actual relative abundance of C^{13} and C^{12} in the carboxyl group because of the incomplete decarboxylation, and would lead to a small error in the calculation of the relative reaction rates of the C^{12} and C^{13} carboxyl anthranilic acid.* This difficulty did not arise, however, since no significant isotope effect was observed in the reaction. Samples of gas from partial thermal, aqueous, and acid catalyzed decarboxylations were analyzed to determine the C^{13} isotope effect. All the mass spectrometer results are given in Table IV. Since there is a probable error of about 0.1% in the individual mass spectrometer analyses, the maximum difference of 0.5% observed in the C^{13}/C^{12} ratios indicates that any isotope effect in this reaction is an order of magnitude smaller than the effect found in all decarboxylations so far reported.

DISCUSSION

Whenever a carbon–carbon bond rupture is the rate determining step in a reaction, an isotope effect is expected to occur if isotopic atoms are directly involved in the bond rupturing.** On this basis, when an acid decarboxylates by a unimolecular mechanism, there should be an isotope effect. For example, an isotope effect has been observed in the decarboxylation of trichloroacetic acid (1) which is known to decompose by a unimolecular process (12). The fact that there is no observed isotope effect in the decarboxylation of anthranilic acid is evidence that the mechanism is not unimolecular.

In 1948, Schenkel and Schenkel-Rudin (9) suggested that some organic acids are decarboxylated by a bimolecular electrophilic substitution mechanism (S_E2). Since then, several examples of S_E2 mechanisms have been found (4,6,10). The acid catalysis and base inhibition observed in our work indicate a bimolecular mechanism for the decarboxylation of anthranilic acid. However, an isotope effect has been observed in the decomposition of mesitoic acid (3,11) which has been shown to decompose by a bimolecular process (10). In the mesitoic decarboxylation, the carbon–carbon bond cleavage must be the rate controlling step to account for the isotope effect. The mechanism proposed by Schubert (10),



For a discussion of the calculation of isotope effects from experimental data, see for example Bigeleisen, J. Science, 110:14. 1949; and Bigeleisen, J. and Allen, T. L. J. Chem. Phys. 19:760. 1951.

***For a theoretical treatment of isotope effect, see Bigeleisen, J. J. Chem. Phys. 17:675. 1949.*

does satisfy this condition. It is assumed that the formation of the protonated acid (or the solvated ion) is reversible with a low activation energy, and that the carbon–carbon bond cleavage is rate controlling.

For the anthranilic acid decarboxylation, two possible points of attack by the proton should be considered. The attack may be on a carboxyl oxygen as proposed for mesitoic acid, or the attack may be on the α carbon as has been suggested by Brown and co-workers for the decarboxylation of *o*-hydroxybenzoic acid (4). Also, several species of anthranilic acid must be considered. Bjerrum (2) has shown that un-ionized $NH_2C_6H_4CO_2H$ molecules exist in solution to a considerable extent as well as the zwitterions $NH_3^+C_6H_4CO_2^-$. Two ionic forms $NH_3^+C_6H_4CO_2H$ and $NH_2C_6H_4CO_2^-$ also exist, their amounts depending on the hydrogen ion concentration.

There are four equilibria mutually dependent.

1. $NH_2 \cdot R \cdot CO_2H \rightleftharpoons NH_2 \cdot R \cdot CO_2^- + H^+$.
2. $NH_3^+ \cdot R \cdot CO_2H \rightleftharpoons NH_2 \cdot R \cdot CO_2H + H^+$.
3. $NH_3^+ \cdot R \cdot CO_2H \rightleftharpoons NH_3^+ \cdot R \cdot CO_2^- + H^+$.
4. $NH_2 \cdot R \cdot CO_2H \rightleftharpoons NH_3^+ \cdot R \cdot CO_2^-$.

(where $R \equiv C_6H_4$)

If we use square brackets to indicate concentrations, then $[NH_2 \cdot R \cdot CO_2^-] \times [H^+] = K_1 [NH_2 \cdot R \cdot CO_2H]$. Because $NH_2C_6H_4CO_2H$ is a weak acid, $[NH_2RCO_2^-] \times [H^+] \cong K_1'$. The decarboxylation rate will be given by the expression, rate $= k [H^+]$ [organic acid species]. Thus, if the ion $NH_2RCO_2^-$ is the reactant, the reaction rate should remain practically constant as the mineral acid concentration is changed. Since, however, the rate increases as the sulphuric acid concentration increases up to 1*N*, this species is not likely the reactant. From 2, $[NH_3^+RCO_2H] \cong K_2' [H^+]$. The reaction rate should thus vary almost directly as the square of the sulphuric acid concentration, and certainly never decrease, if $NH_3^+RCO_2H$ is the reactant. Since the reaction rate decreases when the sulphuric acid concentration increases above 1*N*, this species is also almost certainly eliminated as the reactant.

With either the neutral molecule or the zwitterion as reactant, the acid catalyzed runs are explicable. At low mineral acid concentrations, the increase in hydrogen ion concentration caused by an addition of mineral acid is *relatively* greater than the decrease in either zwitterion or neutral molecule concentration resulting from the addition of the mineral acid. The reaction rate is thus increased by small additions of mineral acid. However, when the hydrogen ion concentration approaches that of the anthranilic acid, Reaction (3) becomes important. $NH_3^+ \cdot R \cdot CO_2H$ is a relatively strong acid owing to the inductive effect of the NH_3^+ group. The concentration of $NH_3^+ \cdot R \cdot CO_2H$, therefore, will become appreciable only at high hydrogen ion concentrations where the concentration of the zwitterion $NH_3^+ \cdot R \cdot CO_2^-$ and consequently of the $NH_2 \cdot R \cdot CO_2H$ in equilibrium with it are both decreased by Reaction (3). At the same time, hydrogen ions are withdrawn from the solution by Reaction (3). It is thus quite conceivable that owing to removal of $NH_3^+ \cdot R \cdot CO_2^-$ and $NH_2 \cdot R \cdot CO_2H$ and a corresponding number of hydrogen ions by the same

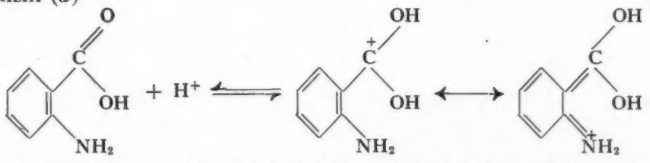
reaction, the reaction rate could decrease with increasing mineral acid addition at some high mineral acid concentration. Hence the reaction rate first increases and then decreases as the sulphuric acid concentration is increased. The acid catalyzed runs are of no help, however, in deciding between the neutral molecule and the zwitterion, since the ratio of their concentrations should be practically insensitive to hydrogen ion concentration.

The possible reaction mechanisms to be considered then are: (*a*) proton attack on the oxygen of the neutral molecule, (*b*) proton attack on the α carbon of the neutral molecule, (*c*) proton attack on the oxygen of the zwitterion, (*d*) proton attack on the α carbon of the zwitterion. Obviously, single proton attack on the oxygen of the zwitterion would not lead to decarboxylation, so Path (*c*) is eliminated.

It is difficult to decide in favor of (*a*), (*b*), or (*d*) as the detailed mechanism. If the reaction goes by Mechanism (*a*) or (*b*) involving the neutral molecule, one might expect *p*-aminobenzoic acid to react faster than anthranilic since the ratio of neutral molecules to zwitterions is greater in *p*-aminobenzoic than in *o*-aminobenzoic acid solutions (2). However, the rate for *p*-aminobenzoic is only about one-half the rate for *o*-aminobenzoic (7). Thus, on this basis, (*d*) would seem to be the preferred mechanism.

In an attack by a proton on the oxygen, Mechanism (*a*), the bond between the ring carbon and carboxyl carbon would tend to increase in double bond character, and hence in bond strength, through the electromeric effect of the *o*-amino group.

Mechanism (*a*)



A high activation energy should then be required for the carbon–carbon bond rupture and the cleavage could reasonably be expected to be rate controlling. Mechanism (*a*) would therefore likely show an isotope effect. In the mesitoic acid case, steric hindrance tends to prevent the planar configuration necessary for resonance interaction of the protonated carboxyl group and the ring, thus lowering the activation energy for bond rupture and favoring this mechanism. But even there, the activation energy must be sufficiently high so that the carbon–carbon bond rupture is rate controlling and an isotope effect results. Thus Mechanism (*a*) is discounted because no isotope effect is obtained with anthranilic acid.

In the neutral molecule, there is a high electron density available at the α carbon because of the mesomeric effect of the ortho amino group. This is not true in the zwitterion where the inductive effects of the NH_3^+ and CO_2^- groups probably balance each other fairly closely, and no mesomeric effect is present. Thus the attack of a proton on the α carbon of the zwitterion,

Mechanism (*d*), should require a higher activation energy than a similar attack on the neutral molecule. It should now be remembered that many acids decarboxylate as zwitterions by a unimolecular process.* The activation energy for carbon–carbon bond rupture in the zwitterion is thus less than that required in the neutral molecule. Mechanism (*b*) would be much more likely to show an isotope effect than (*d*) on this basis, since (*b*) would require a lower activation energy for proton attack and a higher activation energy for carbon–carbon bond rupture than (*d*).

If it is postulated that the mechanism for the decarboxylation *is* (*d*), that is, a frontside attack of a proton on the α carbon of the zwitterion requiring a high activation energy and being rate controlling, followed by rapid carbon–carbon bond rupture requiring a lower activation energy, the lack of an isotope effect is understandable. Such a two step mechanism is more complex than a normal bimolecular (S_E2) mechanism. It envisions a hypothetical carbonium ion which is stable relative to the activated state through which it passed in its formation and, although unstable to the zwitterion, relatively more unstable towards decarboxylation. The carbonium ion is thought of as being in a shallow energy valley between two energy peaks, the higher representing the activation energy for formation of the carbonium ion, the lower representing the activation energy for decarboxylation. No significant isotope effect, as far as decarboxylation is concerned, should occur in the proton attack on the α carbon because the dependence of the activation energy for this step on the mass of the carboxyl carbon can only be very slight. It follows that if there is no isotope effect in the rate controlling step, there will be no isotope effect in the over-all reaction.

Thus, while Mechanisms (*a*) and (*b*) cannot be completely excluded, Mechanism (*d*), which best explains the lack of isotope effect, is considered as being most probable. At the very least, one concludes from the experimental evidence that the decarboxylation is bimolecular, with the attack of a proton somehow being the rate controlling step.

Although the mechanism proposed might appear to apply only to the acid catalyzed reaction, it is equally applicable to the uncatalyzed aqueous and the thermal decarboxylations. In solution, with no added mineral acid, the anthranilic acid itself, by ionization, supplies the protons for the reaction. Thermal decarboxylation only occurs when the anthranilic acid is in the molten state where again the acid can supply the necessary protons.

REFERENCES

1. Bigeleisen, J. and Allen, T. L. J. Chem Phys. 19: 760. 1951.
2. Bjerrum, N. Z. physik. Chem. 104: 147. 1923.
3. Bothner-By, A. A. and Bigeleisen, J. J. Chem. Phys. 19: 755. 1951.
4. Brown, B. R., Hammick, D. Ll., and Scholefield, A. J. B. J. Chem. Soc. 778. 1950.
5. Graham, R. L., Harkness, A. L., and Thode, H. G. J. Sci. Instruments, 25: 119. 1947.
6. Johnson, W. S. and Heinz, W. E. J. Am. Chem. Soc. 71: 2913. 1949.
7. McMaster, L. and Shriner, R. L. J. Am. Chem. Soc. 45: 751. 1923.
8. Pawlewski, Br. Ber. 37: 592. 1904.
9. Schenkel, M. and Schenkel-Rudin, H. Helv. Chim. Acta, 31: 514. 1948.
10. Schubert, W. M. J. Am. Chem. Soc. 71: 2639. 1949.
11. Stevens, W. H., Pepper, J. M., and Lounsbury, M. J. Chem. Phys. 20: 192. 1952.
12. Verhoek, F. H. J. Am. Chem. Soc. 56: 571. 1934.

**For an excellent review of decarboxylation, see Brown, B. R. Quart. Revs. 5:131. 1951.*

THE METHOXYMERCURATION OF 2-METHYL-1-PHENYLPROPENE-1[1]

By L. Berman, R. H. Hall, R. G. Pyke, and George F Wright

ABSTRACT

The addition of the elements of methoxymercuric acetate to 2-methyl-1-phenylpropene-1 occurs to give 2-methyl-2-methoxy-1-phenylpropylmercuric acetate. This structure could not be demonstrated by replacement of chloromercuri by halogeno because 2-methyl-1,2-dimethoxy-1-phenylpropane and 2-methyl-2-phenylpropanal or 3-phenylbutanone-2 were obtained instead of the expected halides. Indeed the 2-methyl-2-methoxy-1-phenylpropyl iodide which Tiffeneau, Orekhoff, and Lévy reported as the reaction product of 2-methyl-1-phenylpropene-1, mercuric acetate, and iodine in methanol is yet unknown, since their product actually comprised the di-ether and aldehyde described above. The structure proof of 2-methyl-2-methoxy-1-phenylpropylmercuric chloride has been accomplished by treatment with hydrazine hydrate. The isolation of 2-methoxy-2-methyl-1-phenylpropane and the two diastereomeric 2,5-dimethyl-2,5-dimethoxy-3,4-diphenylhexanes shows that this reaction has proceeded through intermediate formation of the 2-methoxy-2-methyl-1-phenylpropyl radical.

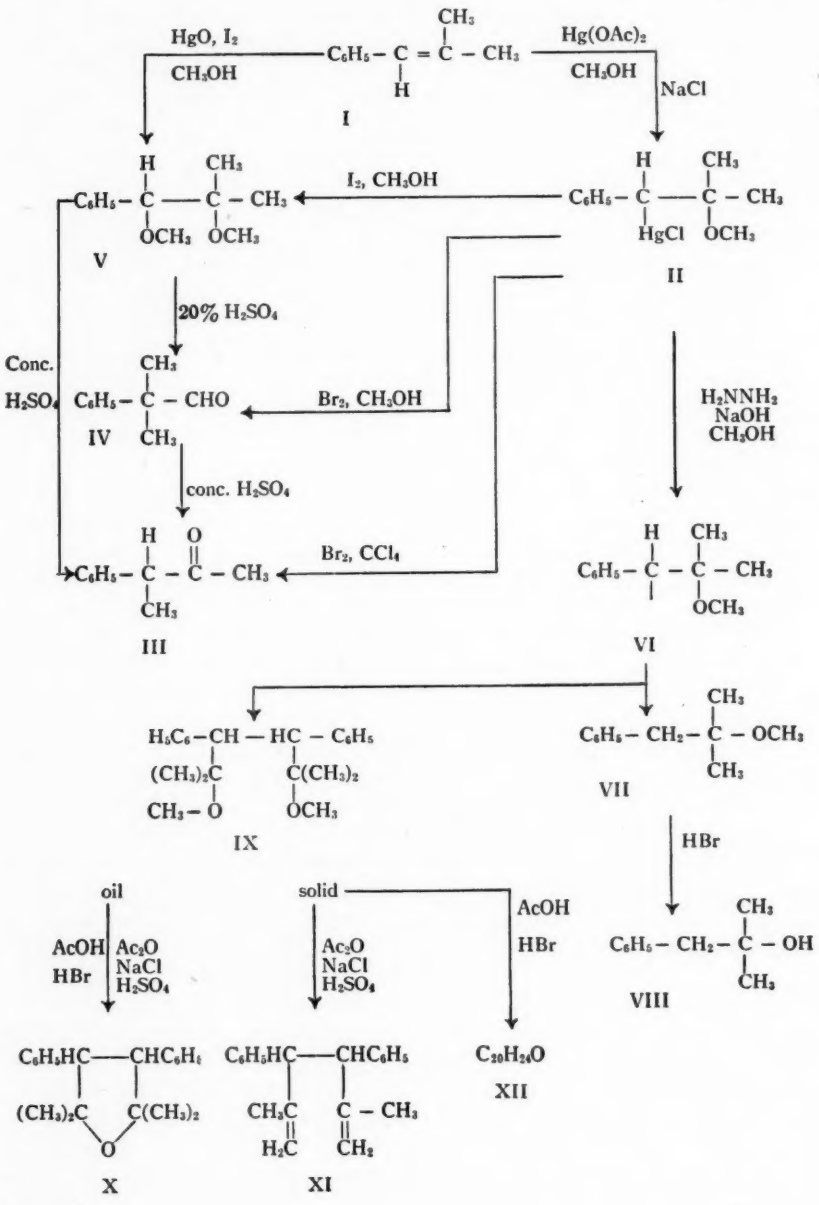
The addition of the elements of methoxymercuric acetate to styrene gives a single product, 2-methoxy-2-phenylethylmercuric salt in high yield (6). The comparable addition to 1-phenylpropene-1 gives a much lower yield of a compound which was designated as 2-methoxy-1-methyl-2-phenylethylmercuric salt (6) on the basis of successive reaction with bromine, alkali, and, finally, acid which produced 1-phenylpropanone-1. The orientation of the addenda is identical with that found when these alkenes are treated with hydrogen chloride. It seemed of interest to extend this study to the methoxymercuration of 2-methyl-1-phenylpropene-1 upon which hydrogen chloride adds oppositely (1).

The methoxymercuration of 2-methyl-1-phenylpropene-1 (I) with mercuric acetate in excess methanol proceeded rapidly in 92% yield. The initial 2-methyl-2-methoxy-1-phenylpropylmercuric acetate was converted to its chloromercuri analogue (II) without having been isolated.

Attempts to demonstrate the structure (II) by halogenation techniques were unsuccessful. When the mercurial was treated with bromine in carbon tetrachloride at 0° a rapid, vigorous reaction occurred, but the sole product was 3-phenylbutanone-2 (III). This ketone (evidently the result of a 1,2-rearrangement) cannot be definitive for structure proof. Indeed it does not even demonstrate the intermediate formation of 2-methoxy-2-methyl-1-phenylethyl bromide, since the action of bromine in methanol on the chloromercurial yields 2-methyl-2-phenylpropanal (IV). This aldehyde is an alternative 1,2-rearrangement product involving interchange of phenyl and methoxy radicals rather than halomercuri (or its equivalent) and methyl radicals to produce the ketone III.

The iodination of the mercurial (II) likewise did not contribute to structure

[1] *Manuscript received January 8, 1952.*
Contribution from Department of Chemistry, University of Toronto, Toronto, Ont.


HgO, I2
CH3OH
C6H5–C(H)=C(CH3)–CH3
I
Hg(OAc)2
CH3OH
NaCl
I2, CH3OH
V
II
20% H2SO4
Conc. H2SO4
IV
Br2, CH3OH
H2NNH2
NaOH
CH3OH
conc. H2SO4
Br2, CCl4
III
VI
H3C6–CH–HC–C6H5
(CH3)2C
CH3–O
C(CH3)2
OCH3
IX
C6H5–CH2–C(CH3)(OCH3)–CH3
VII
HBr
oil
AcOH
HBr
Ac2O
NaCl
H2SO4
solid
Ac2O
NaCl
H2SO4
AcOH
HBr
C6H5–CH2–C(CH3)(OH)–CH3
VIII
C6H5HC–CHC6H5
(CH3)2C
C(CH3)2
O
X
C6H5HC–CHC6H5
CH3C
C–CH3
H2C
CH2
XI
C20H24O
XII

proof, although 2-methoxy-2-methyl-1-phenylethyl iodide supposedly was known (4). Treatment of the mercurial with iodine in methanol yielded instead a compound which we designate as 1,2-dimethoxy-1-methyl-2-phenylpropane (V), contaminated with 2-methyl-2-phenylpropanal, (IV). The dimethoxy compound (V) could be converted by 1,2-rearrangement to the aldehyde (IV) in 20% aqueous sulphuric acid or to 3-phenylbutanone-2 (III) in concentrated sulphuric acid. The latter conversion was expected since IV is isomerized to III in the concentrated acid. These rearrangements do not designate V as intermediate in the conversion of the mercurial (II) to the aldehyde by methanolic bromine since V is stable in this medium.

Tiffeneau and Orekhoff produced the compound they called 2-methoxy-2-methyl-1-phenylpropyl iodide by treatment of 2-methyl-1-phenylpropene-1 with iodine and mercuric oxide in methanol. One might presume that this reaction is related to that of methoxymercuration. We repeated the procedure of Tiffeneau and Orekhoff and found that their product was not the iodide but, instead, the dimethoxy derivative (V) containing about 15% of the aldehyde (IV). They had assumed this product to be the iodide and, without purification, had treated it with alcoholic potassium hydroxide. This treatment did not alter the mixture of IV and V except to remove traces of halogen, but it caused them wrongly to designate their product as 1-hydroxy-2-methoxy-2-methyl-1-phenylpropane. This error was perpetuated by Lévy (3) who effected the same 1,2-rearrangements on the dimethoxy compound (V) which we have described earlier in this report, although she thought she was using the methoxy-alcohol.

The product obtained by iodination of the mercurial (II) in methanol, or by the reaction of Tiffeneau and Orekhoff, cannot be 1-hydroxy-2-methoxy-2-methyl-1-phenylpropane since it gives no gas in the Grignard Machine (2). The isomeric dimethyl acetal of 2-methyl-2-phenylpropanal would, of course, give an identical analysis. However the slow conversion (three hours in 20% aqueous sulphuric acid) of V to the aldehyde (IV) is not characteristic of an acetal. Furthermore, 2,4-dinitrophenylhydrazine does not react with V under conditions effective for conversion of the diethyl acetal of 2-methyl-2-phenylpropanal to its 2,4-dinitrophenylhydrazone.

It was evident from these results that halogenation of the methoxymercurial from 2-methyl-1-phenylpropene-1 could not provide a satisfactory structure proof. We chose, therefore, to utilize a reductive reaction recently reported (5) for replacement of mercury by hydrogen. When the methoxymercurial (II) was treated with hydrazine hydrate in alkaline methanol a 9% yield of 2-methoxy-2-methyl-1-phenylpropane (VII) was obtained. This was identified by demethylation with hydrogen bromide to 2-hydroxy-2-methyl-1-phenylpropane (VIII). Excluding the occurrence of rearrangement the structure of the methoxymercurial must therefore be 1-chloromercuri-2-methoxy-2-methyl-1-phenylpropane (II). The addition of the elements of methoxymercuric acetate to β,β-dimethylstyrene (I) thus occurs oppositely to that found for styrene.

The proof is not vitiated by the low yield of VII because two other products, obtained in 76% yield, confirm the structure allocation. These two products are the diastereomeric 2,5-dimethoxy-2,5-dimethyl-3,4-diphenylhexanes (IX). Together with VII they are the expected products if the reduction of 2-methyl-2-methoxy-1-phenylpropylmercuric chloride (II) proceeds via the intermediate 2-methyl-2-methoxy-1-phenylpropyl radical (VI). This radical cannot be stabilized by loss of a hydrogen atom after the manner in which the 2-methoxycyclohexyl radical becomes 2-methoxycyclohexene (5), and hence must dimerize unless it can acquire a hydrogen atom (from hydrazine) in order to become VII.

The diastereomeric configurations of the solid and oil (IX) have not yet been ascertained. Structural identification has been accomplished by demethylation. The oily diastereomer which is formed in preponderance may be demethylated by hydrogen bromide in acetic acid, or by sulphuric acid and sodium chloride in acetic anhydride. The product is a solid which is inert toward Grignard reagent, alkaline potassium permanganate, acid dinitrophenylhydrazine, and concentrated sulphuric acid. According to its elemental analysis these properties would define the compound as 2,2,5,5-tetramethyl-3,4-diphenyltetrahydrofuran (X).

In contrast the demethylation of the solid diastereomer (IX) does not proceed smoothly. When it is heated with hydrobromic and acetic acids a poor yield is obtained of a compound which seems to be isomeric with X. However its properties are quite different. It reacts with bromine although no definite product has been isolated. A structure has not been assigned to this compound, and it is designated tentatively as XII.

When the solid diastereomer (IX) is demethylated with sodium chloride plus sulphuric acid in acetic anhydride a mixture is obtained comprising a yet-unidentified compound containing sulphur, together with a hydrocarbon. The analysis of this hydrocarbon designates it as 2,5-dimethyl-3,4-diphenylhexadiene-1,5 (XI) since formaldehyde is formed from it by ozonolysis.

The authors are grateful to J. K. Whitely for the X-ray diffraction measurements.

EXPERIMENTAL*

1-Chloromercuri-1-phenyl-2-methoxy-2-methylpropane (II)

A solution of 13.2 gm. (0.1 mole) of 2-methyl-1-phenylpropene-1 and 31.8 gm. (0.1 mole) of mercuric acetate in 250 ml. of methanol was allowed to stand at 25° for 15 hr. After 40 ml. of 10% aqueous sodium hydroxide was added, the solution was filtered into 400 ml. of 2% aqueous sodium chloride. The precipitate was filtered after four hours. It weighed 36.6 gm. (92% of theory), m.p. 78–79.5°. Four crystallizations from absolute ethanol (2 ml. per gm.) raised this m.p. to 81–81.5°. Calcd. for $C_{11}H_{15}OHgCl$: C, 33.0; H, 3.78; OCH_3 7.76; Found: C, 33.2; H, 3.62; OCH_3, 7.78.

**All melting points have been corrected against reliable standards.*

1,2-Dimethoxy-1-phenyl-2-methylpropane (*V*)

A. Method of Tiffeneau and Orekhoff

To a solution of 15 gm. (0.114 mole) of 2-methyl-1-phenylpropene-1 in 125 ml. of methanol was added with cooling 30 gm. (0.118 mole) of iodine and 24.6 gm. (0.114 mole) of yellow mercuric oxide. Moderate heat evolution occurred together with precipitation of mercuric iodide. After 40 hr. the solvent was evaporated in an air draft. The black residue, diluted with 40 ml. of diethyl ether, was filtered and the filtrate washed with a concentrated aqueous solution of potassium iodide containing enough sodium bisulphite to decolorize the nonaqueous layer. It was then washed successively with water, with 5% aqueous sodium hydroxide until no trace of mercuric salt was evident, then with water, and finally repeatedly with concentrated aqueous sodium bisulphite. The solution was dried with magnesium sulphate and distilled under 10 mm. Four fractions boiling from 94–104° showed n_D^{20} from 1.505 to 1.497. Grignard Machine analyses gave no active hydrogen but a carbonyl value indicating contamination to the extent of about 10% with 2-methyl-2-phenylpropanal (n_D^{20} 1.5147) and this was confirmed by the methoxyl content of 29.1%. Purification was effected by treatment for one day with ethanolic hydroxylamine. After aqueous alkali was added *V* was extracted with ethyl ether and distilled, b.p. 164–167° (19 mm.), n_D^{20} 1.4991, d_4^{20} 0.984. The entire yield (15.4 gm.) is 70% of theoretical. Calcd. for $C_{12}H_{18}O_2$: C, 74.1; H, 9.36; OCH_3, 31.9. Found: C, 74.3; H, 9.07; OCH_3, 31.7.

B. From Mercurial II with Iodine in Methanol

A solution of 30 gm. (0.075 mole) of 1-chloromercuri-2-methoxy-2-methyl-1-phenylpropane in 150 ml. of methanol was treated with 19.1 gm. (0.075 mole) of iodine during 15 min. with initial moderate cooling. After five hours the mixture was processed as described in Procedure *A*. A 75% crude yield (10.9 gm.) was obtained, b.p. 110–115° at 12 mm., n_D^{20} 1.5004. According to the Grignard Machine analysis this product contained about 15% of 2-methyl-2-phenylpropanal.

2-Methyl-2-phenylpropanal

A. From 1,2-Dimethoxy-2-methyl-1-phenylpropane

The method of Lévy (3) was repeated without incident to give the aldehyde, identified as its semicarbazone, m.p. 182–182.5°. Calcd. for $C_{11}H_{15}N_3O$: C, 64.4; H, 7.32; N, 20.5. Found: C, 63.9; H, 7.28; N, 20.4.

B. From Mercurial, (II)

A mixture of 17.4 gm. (0.0437 mole) of the mercurial and 100 ml. of methanol was chilled by ice-water while 7.05 gm. (0.044 mole) of bromine was added during 10 min. After eight hours at 25° the clear solution was evaporated by an air draft. A solution of the dark residue in 20 ml. of carbon tetrachloride was filtered, washed with 5% aqueous sodium hydroxide until no more mercuric salt was evident, and then washed thrice with water. The solution was distilled, finally at 100–103° (14 mm.), wt. 4.0 gm. (80% of theory), n_D^{20} 1.5147. This product gave a semicarbazone, m.p. 176–178°, which (after two crystallizations from ethanol) was found by mixed melting point to be identical with that

prepared by Procedure *A*.

2-Phenylbutanone-2

A. From 1,2-Dimethoxy-2-methyl-1-phenylpropane (V)

Lévy's method was used with the substance identified by analysis and by lack of active hydrogen to be the dimethoxy derivative. The ketone was identified as the semicarbazone, m.p. 178–178.5°. Calcd. for $C_{11}H_{15}N_3O$: C, 64.4; H, 7.32; N, 20.5. Found: C, 63.9; H, 7.40; N, 19.9.

B. From Mercurial, (II)

A solution of 25 gm. (0.063 mole) of the mercurial in 120 ml. of carbon tetrachloride was chilled in an ice bath while 10.1 gm. (0.063 mole) of bromine was added during 10 min. Decolorization was almost instantaneous. After filtration the solution was evaporated *in vacuo* to a volume of 30 ml. The residual solution was washed with 5% aqueous sodium hydroxide until there was no evidence of mercuric salt, then thrice with water. The solution was distilled, finally at 14 mm., b.p. 98–101°, wt. 7.3 gm. (79.5% yield), n_D^{20} 1.5163. The semicarbazone was prepared, m.p. 176–178° and characterized by mixed melting point.

Reduction of Mercurial, (II), by Hydrazine Hydrate

To a solution of 39.8 gm. (0.1 mole) of mercurial (II) in 200 ml. of methanol was added 20 ml. (0.20 mole) of 40% aqueous sodium hydroxide and 2.0 ml. (0.04 mole) of 85% hydrazine hydrate. The mixture was boiled under reflux for 90 min. Then another 2 ml. of hydrazine hydrate was added and heating was continued for 12 hr. The solution was then cooled and filtered after precipitation was complete. The precipitate was extracted with 120 ml. of boiling ethanol. When the extract was cooled it yielded 4.7 gm. of 2,5-dimethyl-2,5-dimethoxy-3,4-diphenylhexane, m.p. 135°. Crystallization from 95% ethanol (10 ml. per gm.) raised this m.p. to 139–139.8°. Calcd. for $C_{22}H_{30}O_2$: C, 81.0; H, 9.25; OCH_3, 19.0. Found: C, 81.4; H, 9.37; OCH_3, 18.5.

The original methanolic filtrate was concentrated *in vacuo*, then diluted with water and extracted with ether. This extract was washed with water, dried with magnesium sulphate, and distilled under 15 mm. The first fraction, b.p. 87–93°, n_D^{20} 1.5089, wt. 2.2 gm., was filtered to remove mercury and redistilled as 1.45 gm. of 2-methoxy-2-methyl-1-phenylpropane, (VII) (9% of theory), b.p. 87–90° (15 mm.), n_D^{20} 1.5007. Since this product was contaminated slightly with benzaldehyde and 2-methyl-1-phenylpropene-1 it was again distilled, b.p. 86° (10 mm.), n_D^{20} 1.4980. Calcd. for $C_{11}H_{16}O$: C, 80.5; H, 9.85; OCH_3, 18.9. Found: C, 80.9; H, 9.77; OCH_3, 18.5.

After removal of VII by distillation a second fraction, b.p. 198–205° (15 mm.), wt. 7.7 gm., n_D^{20} 1.5406, was obtained. After several days this solidified partially. Filtration removed the crystals, m.p. 108–125°. After crystallization from ethanol this was found to be more of the solid 2,5-dimethyl-2,5-dimethoxy-3,4-diphenylhexane. Total yield was 6.2 gm. or 38% of the theoretical amount.

The remaining oil, which did not crystallize further, is thought to be largely

the diastereomeric 2,5-dimethyl-2,4-dimethoxy-3,4-diphenylhexane. The yield was 6.2 gm. or 38% of theoretical. It was redistilled, b.p. 200–210° (13 mm.), n_D^{20} 1.54345. Calcd. for $C_{22}H_{30}O_2$: C, 81.0; H, 9.25; OCH_3, 19.0. Found: C, 81.1; H, 9.14; OCH_3, 18.9.

2-Methyl-1-phenylpropyl Phenylcarbamate

A solution of 0.5 gm. (0.003 mole) of 2-methoxy-2-methyl-1-phenylpropane and 2 ml. (0.015 mole) of 48% hydrobromic acid in 4 ml. of acetic acid was warmed on the steam bath for two hours. After cooling, the mixture was made alkaline and steam distilled. The first 10 ml. of distillate was heated with 1.3 gm. (0.009 mole) of potassium carbonate for 20 hr. on a steam bath. The cooled mixture was extracted with ether. The extract, dried with magnesium sulphate, was concentrated to 0.75 ml. volume and then was treated with 0.2 ml. of dry pyridine and 0.1 ml. of phenylisocyanate. This treatment yielded 0.12 gm. (55% yield) of urethane, m.p. 90–91°. After two crystallizations from 95% ethanol (1 ml. per gm.) the identity of the compound (m.p. 92°) was demonstrated by mixed melting point with an authentic sample (1).

2,2,5,5,-Tetramethyl-3,4-diphenyltetrahydrofuran (X)

A. From Hydrobromic and Acetic Acids

A solution of 1.0 gm. (0.003 mole) of 2,5-dimethoxy-2,5-dimethyl-3,4-diphenylhexane (liquid diastereomer, IX) and 3.0 ml. (0.023 mole) of 48% hydrobromic acid in 10 ml. of glacial acetic acid was heated on the steam bath for 150 min. The solution was cooled, neutralized cold with 10% aqueous sodium hydroxide, and thrice extracted with ether. This ether solution was evaporated to a volume of 2 ml. and chilled to cause precipitation of 0.5 gm. (59% of theoretical), m.p. 84–86°. The filtrate was distilled at 183–187° (12 mm.); the semisolid distillate was dissolved in hot petroleum ether (b.p. 60-70°C.) and thus yielded 20% more product, m.p. 95–97°. The evaporated mother liquors dissolved to a blood-red solution in concentrated sulphuric acid. Precipitation into ice gave a further 4% of impure product.

The crude product was crystallized from 95% ethanol (2 ml. per gm.) and from petroleum ether (b.p. 60–70°, 5 ml. per gm.) and then melted at 101.4–101.6°C. The X-ray diffraction powder pattern was determined (CuK_α, Ni filtered radiation) at relative intensities $[I/I_0]$ for spacings (Å) as follows: [10] 4.09; [9] 4.57; [7] 5.90; [4] 7.62, 6.70; [3] 3.75, 3.46, 3.23; [2] 2.92, 2.61; [1] 2.27, 2.09, 1.90. Anal. calcd. for $C_{20}H_{24}O$: C, 85.9; H, 8.67. Found: C, 85.8; H, 8.62.

B. From Sodium Chloride – Sulphuric Acid in Acetic Anhydride

A solution of 1.00 gm. (0.003 mole liquid diastereomer, IX) in 10 ml. of acetic anhydride was chilled to 0°C. To this solution was added 1 gm. of sodium chloride and 0.1 ml. of 96% sulphuric acid. The mixture was shaken for 10 hr., then poured into ice, neutralized with 40% aqueous alkali, and boiled for 20 min. After cooling, the suspension was extracted twice with ether and twice with acetonitrile. After the accumulated extracts were dried with magnesium they were distilled, finally at 160–180° (10 mm.). When this distillate (0.61 gm.) was diluted with 1 ml. of ethanol, 0.1 gm. (12% of theoreti-

cal yield) of X, m.p. 92°, was obtained. This was identified by mixed melting point after crystallization from ethanol.

Demethylation of Solid 2,5-Dimethoxy-2,2,5,5-tetramethyl-3,4-diphenylhexane (IX)

A. With Hydrobromic and Acetic Acids

When this solid diastereomer was treated like the liquid diastereomer with these reagents the product (0.4 gm.) distilled at 178–192° (11 mm.). When it was diluted with 0.5 ml. of petroleum ether (b.p. 40–60°) the crystals weighed 0.05 gm. and melted at 78–86°. Crystallization from ethanol raised this melting point to 91°C. Anal. calcd. for $C_{20}H_{24}O$: C, 85.9; H, 8.67. Found: C, 85.4; H, 9.16. The X-ray diffraction powder pattern was determined using CuK_α radiation (Ni filtered). Relative intensities at *d* spacings in Å are: [10] 4.98; [9] 4.72; [7] 5.82; [5] 7.52; [3] 2.06; [2] 3.91, 3.36, 2.79; [1] 4.19, 3.77, 2.43. A mixed melting point with 2,2,5,5,-tetramethyl-3,4-diphenyltetrahydrofuran was lowered to 60°C.

B. With Sodium Chloride – Sulphuric Acid in Acetic Anhydride

A solution of 1.00 gm. (0.003 mole, solid diastereomer, IX) in 10 ml. of acetic anhydride was treated at 0° with 1 gm. of sodium chloride and 0.1 ml. of 96% sulphuric acid. After shaking for 10 hr. the whole was poured into ice and neutralized with 40% aqueous alkali. The gummy precipitate was filtered with difficulty and dissolved in hot ethanol. The crystals of 2,5-dimethyl-3,4-diphenylhexadiene-1,5 (XI) weighed 0.1 gm. (12% of theoretical yield) and melted at 163.8-164.4°. This was recrystallized from hot ethanol (4 ml. per gm.), m.p. unchanged. Calcd. for $C_{20}H_{22}$: C, 91.5; H, 8.47. Found: C, 91.8; H, 8.60. The X-ray diffraction powder pattern was determined using CuK_α radiations (Ni filtered). Relative intensities $[I/I_0]$ for *d* spacings in Å are: [10] 5.90; [8] 3.81; [6] 3.09, 4.13; [5] 9.02, 6.65, 5.29, 3.57, 3.48; [4] 5.62, 3.64; [2] 1.98; [1] 3.38, 2.78, 2.68, 2.02; [0.5] 4.95, 2.39, 2.31, 2.15; [0.25] 4.54. The compound XI was insoluble in cold 96% sulphuric acid. When treated with bromine in carbon tetrachloride, hydrogen bromide was evolved.

Evaporation of the ethanolic solution from which XI was isolated left a residue which was dissolved in petroleum ether (b.p. 60–70°). A microcrystalline solid precipitated after five days, 0.10 gm., m.p. 194–196°C. This was crystallized thrice from ethyl acetate, m.p. 224–225°C. Anal. found: C, 61.8; H, 6.26; S, 3.78. The powder diffraction pattern, determined with CuK_α radiations (Ni filtered) showed relative intensities $[I/I_0]$ at *d* spacings in Å as follows: [10] 13.20; [6] 17.31; [5] 6.78; [4] 5.77; [3] 5.35, 1.99; [2] 6.34, 4.98, 4.73, 4.20, 3.83; [1] 9.35, 7.72, 3.11.

The compound was formed also by treatment of XII with concentrated sulphuric acid. The reddish-brown solution after 15 min. was poured into ice and the precipitate was filtered off, washed with ether, and dried. A mixed melting point showed that it was identical with the compound melting at 224–225°. On the other hand reflux of the sulphur-containing compound with 10% aqueous sodium hydroxide for two hours gave a suspension which was

chilled and filtered. The solid melted at 80°C. A mixed melting point with XII was not lowered. The aqueous filtrate, when acidified with hydrochloric acid, yielded a precipitate with aqueous barium chloride.

Ozonolysis of 2,5-Dimethyl-3,4-diphenylhexadiene-1,5 (XI)

A solution of 0.15 gm. (5.75×10^{-4} mole) of XI in 7 ml. of ozonized and purified carbon tetrachloride was treated with 10 liters of 5% ozonized oxygen at 0° during 2.5 hr. The solid ozonide was hydrolyzed with 20% acetic acid and 60 mgm. of zinc dust in the carbon tetrachloride medium at 40–50° in a stream of nitrogen. No acetone could be detected in the effluent gas by methanolic dinitrophenylhydrazine. The aqueous layer (7 ml.) was distilled almost completely, then neutralized and treated with 30 ml. of a saturated aqueous solution of dimethyldihydroresorcinol. The precipitate, methylene-*bis*-dimethyldihydroresorcinol weighed 0.12 gm. (35% of the theoretical yield), m.p. 189°. A mixed melting point with an authentic specimen was not lowered. The chloroform layer was evaporated to leave an oil which gave a precipitate with methanolic dinitrophenyl hydrazine – hydrogen chloride reagent.

REFERENCES

1. HALL, R. H., PYKE, R. G., and WRIGHT, G. F. J. Am. Chem. Soc. (In press.)
2. KOHLER, E. P., STONE, J. F. Jr., and FUSON, R. C. J. Am. Chem. Soc. 49: 3181. 1927.
3. LÉVY, J. Bull. soc. chim. France (Ser. 4), 29: 820. 1921.
4. TIFFENEAU, M. and OREKHOFF, A. Bull. soc. chim. France (Ser. 4), 29: 809. 1921.
5. WRIGHT, G. F. To be published.
6. WRIGHT, G. F. J. Am. Chem. Soc. 57: 1993. 1935.

COEXISTENCE PHENOMENA IN THE CRITICAL REGION

I. THE GRAVITY EFFECT IN ETHANE FROM LIGHT SCATTERING[1]

By F. E. Murray[2] and S. G. Mason

ABSTRACT

A method of measuring density gradients caused by gravitational compression in gases near the critical region is described. Relative light scattering coefficients of ethane in bombs 40 cm. long increase, remain constant, or decrease in the downward direction, depending upon the mean filling density. These observations have been correlated with the variation of scattering coefficient with density, and show that appreciable density gradients exist. These observations are confirmed by parallel studies of condensation, and serve to explain the flat portion of the liquid–vapor coexistence curve found with a carefully purified system.

INTRODUCTION

The variation of pressure with height in an isothermal fluid is given by the equation

$$\left(\frac{\partial p}{\partial x}\right)_T = \rho g = \frac{g}{v} \tag{1}$$

where x is the vertical distance (downwards) from a datum,

and ρ and v respectively are the density and specific volume. Using the transformation

$$\left(\frac{\partial p}{\partial x}\right)_T = \left(\frac{\partial p}{\partial v}\right)_T \left(\frac{\partial v}{\partial x}\right)_T$$

and introducing the isothermal compressibility $\beta = -\frac{1}{v}\left(\frac{\partial v}{\partial p}\right)_T$, one can write Equation (1) in the form:

$$-\left(\frac{\partial v}{\partial x}\right)_T = \frac{1}{\rho^2}\left(\frac{\partial \rho}{\partial x}\right)_T = g\beta. \tag{2}$$

Near the liquid–vapor critical point, at which $\beta \to \infty$, Equation (2) indicates that large vertical density gradients can occur. Ruedy (13) has attempted to estimate their magnitudes from equations of state but the values given are open to doubt. The most reliable values of β near the critical point are those of MacCormack and Schneider (7) calculated from p-v-T measurements on sulphur hexafluoride. Using their value of $\beta = 12.8$ atmos.$^{-1}$ at $\Delta T = (T - T_c) = 0.003$°C. (the lowest gaseous temperature for which β could be evaluated) and $\rho_c = 0.73$ gm. per cc., the density gradient calculated from Equation (2) is 0.9 % per cm. For a methane–propane mixture of unstated ΔT, Blosser and Drickamer (3) report $\beta = 112$ atmos.$^{-1}$ at $\rho = 0.21$ gm. per cc., a value derived from light scattering measurements. The calculated gradient for this case is 2.4% per cm.

[1] *Manuscript received February 20, 1952.*
Contribution from Chemistry Department, McGill University, Montreal, Que.
[2] *Holder of a Studentship from the National Research Council of Canada.*

Until recently, the importance of these gradients in interpreting some of the anomalies revealed by refined measurements in the critical region has been underestimated. It now appears that the "flat top" of the liquid–vapor coexistence curve at the critical temperature, which has been the subject of some controversy (12) but whose existence has recently been confirmed in a careful series of measurements on ethane in this laboratory (17) and on xenon by Weinberger and Schneider (16), has its origin in the gravity effect, and may vanish when the height of the system under study is reduced to zero. This behavior accords with the classical theory of the critical point, and the recently revised views of Mayer (9, 18) on the possible existence of a unique critical point.

Direct measurement of such density gradients is difficult. Atack and Schneider (1), however, have found that equilibrium gradients in the activity of solutions of chloroiodobenzene (containing radioactive iodine) in ethane existed above the critical temperature. An alternative method, which is based upon measurements of the vertical variation in light scattering, is employed in the investigation described here; this has the advantage of being applicable to one-component systems.

For the intensity of scattered radiation, Einstein and Smoluchowski (11) derived a relation which may be expressed in the form

$$I = I_0 \frac{A}{\lambda^4} \left(\frac{\partial \epsilon}{\partial \rho}\right)_T^2 \rho^2 T\beta \tag{3}$$

where I_0, I are incident and scattered intensity repectively,

A is a constant depending upon the geometry of the optical arrangement,

λ is the wave length,

and ϵ the optical dielectric constant.

With the assumption that $(\partial\epsilon/\partial\rho)_T$ is constant over a short density range, then at fixed I_0, λ, and T, Equation (3) may be simplified to

$$I = B\rho^2\beta \tag{4}$$

where B is a constant.

Combining Equations (2) and (4) gives

$$\left(\frac{\partial \rho}{\partial x}\right)_T = g\frac{I}{B}.$$

Thus if B and I are known, it is possible to calculate $(\partial\rho/\partial x)_T$. If I is measured over a range in heights, the corresponding change in ρ can be evaluated from

$$\rho_x - \rho_0 = \int_0^x \frac{g}{B} I(x)dx. \tag{5}$$

This principle, while attractive from a theoretical point of view, has several limitations. It is difficult to construct a high pressure cell of appreciable height,

having a simple optical geometry, and in which the system can be efficiently stirred and accurately thermostatted. Further, the validity of Equation (3) near the critical point may be questioned since it neglects correlations between neighboring density fluctuations which several authors (5, 10) consider to be of significance.

Instead, it was considered preferable to adopt a more direct approach not depending upon the validity of light scattering theory. This method utilizes the observation that near T_c, as ρ is increased isothermally, I increases rapidly, passes through a maximum, and then decreases rapidly (15). The use of a bomb of comparatively simple design made it possible to measure relative scattered intensities as functions of the average filling density and of the height. The scattered intensity in this way served only as a parameter in calculating $(\partial\rho/\partial x)_T$.

In experiments on ethane described below, the existence, above T_c, of appreciable equilibrium gradients was conclusively demonstrated. This was corroborated by visual observation of condensation behavior. Further, it was shown that when equilibrium gradients are destroyed by stirring, there is an appreciable time lag before equilibrium is re-established.

To avoid confusion in terminology, we define T_c to be the reversible temperature at which the meniscus of a critical filling disappears on heating (with stirring) or reappears on cooling. This point has previously been designated as T_s (8). A critical filling is one in which the point of meniscus disappearance (with stirring) or reappearance lies within the confines of the bomb. ΔT and $\Delta\bar{\rho}$ are used to designate $(T - T_c)$ and $(\bar{\rho} - \bar{\rho}_m)$ respectively. Here $\bar{\rho}$ is the mean filling density and $\bar{\rho}_m$ is the density of maximum scattering.

EXPERIMENTAL

Apparatus

The system was enclosed in a long vertical pyrex bomb B (Fig. 1) of 10 mm. I.D. and 3 mm. wall thickness. The contents could be vigorously stirred by a small perforated steel plug M moved up and down by two alnico magnets on a guide rod (Fig. 1*b*).

The bomb B was flanged at one end and connected to an Aminco super-pressure metal filling system by means of an adaptor and gasket G (Fig. 1*b*). The metal system was connected through the valve V_3 to a low pressure glass filling and storage system. The volume was varied by means of a mercury injector P (Fig. 1*a*). In this way, a single sample could be studied over a continuously variable range of densities, thus eliminating complications arising from variable composition of different samples. A revolution counter was attached to the screwdrive mechanism of the injector piston so that desired volume settings could be reproduced and calculated by reference to a known volume datum marked on the bomb.

Using a sensitive galvanometer with a calibrated shunt, measurements of transverse scattered intensity were made at various levels along the bomb with a 931A photomultiplier tube. This tube was focussed by two slits on a small

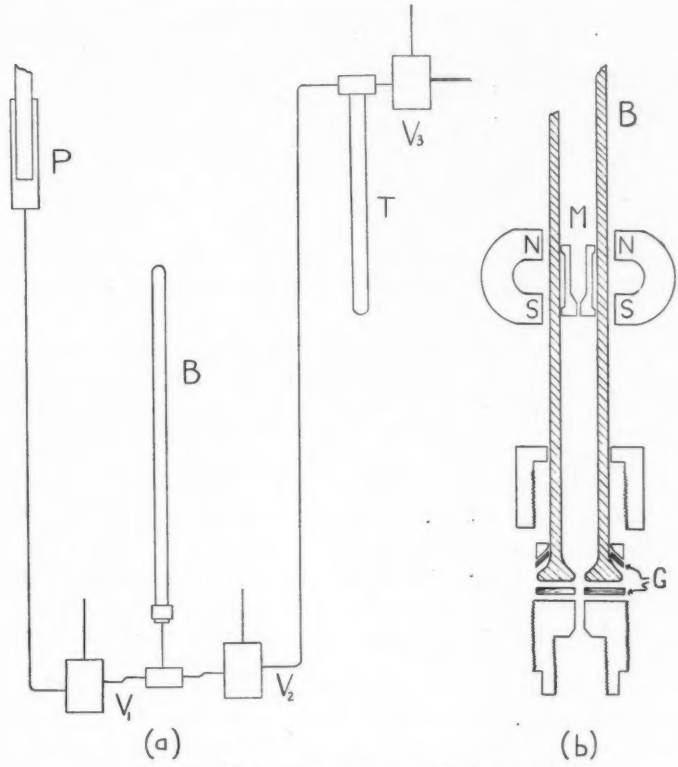


FIG. 1. Glass bomb and pressure filling system.

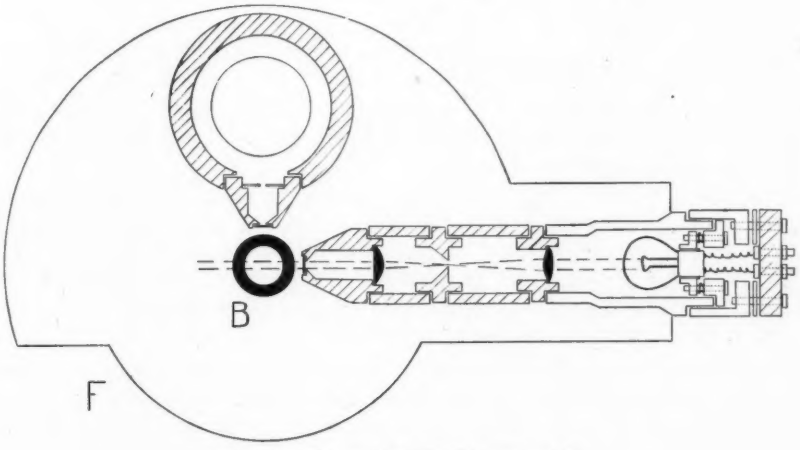


FIG. 2. The optical measuring assembly.

volume element of a beam approximately 2 mm. in diameter from the tungsten filament of a 6 v. bulb (Fig. 2). Power for the tube was regulated by a constant voltage transformer and power supply as used by Hadow *et al.* (4).

The light source and photocell assembly were mounted on a flat plate *F* (Fig. 2) whose position was controlled by a brass bushing through which the bomb passed. In this way, the geometry of the optical assembly with respect to the bomb was fixed. The measuring assembly was raised or lowered along the bomb by means of a calibrated lucite rod.

The metal filling system, mercury injector, bomb, and optical assembly were mounted on a rigid brass insert frame which fitted into a thermostatting tank.

The thermostat tank was of sheet brass and was equipped on each side with a vertical plexiglas window 24 in. long and 4 in. wide. The tank was totally enclosed in an insulated wooden box; the box contained plexiglas windows which were closed off when light scattering measurements were made.

A clear oil (Risella 17) was used as the thermostatting medium; this also served as a lubricant for the moving parts of the system. To remove dust, an arrangement for continuous filtration of the oil through an automobile oil filter was incorporated in the apparatus.

The temperature of the bath could be measured to a differential accuracy for 0.0005°C. by means of a high resistance thermistor (Western Electric No. 14A) in a sensitive Wheatstone bridge circuit. Because of drift, the thermistor was repeatedly calibrated against a Beckmann thermometer. The latter was calibrated against a standard thermometer to an absolute accuracy of ±0.02°C. The bath temperature could, by manual adjustment of the heating elements, be maintained constant within ± 0.001°C. To check for temperature gradients within the bath, the thermistor was mounted on a lucite rod, which was moved to various levels; no measurable gradients were ever detected.

Procedures

Ethane, purified by fractional distillation in the glass filling system, was condensed over liquid air into the steel bomb *T* (Fig. 1). The valve V_3 was then closed and the gas was allowed to expand into the glass bomb *B*; V_2 was closed and the mercury forced from the injector (V_1 open) until the desired density was obtained.

The distance of the light beam from the top of the bomb was measured using a fixed level cathetometer to read the calibrated lucite rod which raised or lowered the optical assembly. Light scattering measurements were recorded at 2 cm. intervals along the bomb. The magnets prevented measurements being taken over the bottom 10 cm. of the bomb since they were mounted to travel below the optical assembly.

Because of the large samples used, it was not practicable to determine the mass of a filling by direct weighing; instead this was done by a gas expansion method which was subject to an error of 3%. The error in the quantity $\Delta\bar{\rho}$ was 2×10^{-5} gm. per cc.

RESULTS

Preliminary Experiments

Great difficulty was experienced in obtaining pure samples of ethane. In the first experiments, technical grade material was purified by fractional distillation, but the liquid–vapor coexistence curves were found to be retrograde (Fig. 4). When fractionally-distilled Phillips Research Grade ethane was used, the effect persisted. It was concluded that the impurities came from the teflon gaskets and valve packings with which the gas came in contact while the bomb was being filled. When a polythene gasket, graphite packing, and Research Grade ethane were used, the retrograde effect disappeared, i.e. the maximum temperature of meniscus reappearance corresponded to a critical filling. This was taken as the criterion of a pure system.

While the initial experiments were largely vitiated by the impurities, a number of interesting observations were made.

The scattered intensity I, expressed in units of galvanometer deflection, represented by Curve A of Fig. 3, was low and the bomb contents appeared visually transparent. Thus, this curve indicates the maximum possible values of spurious scattering from the walls of the bomb; comparison with Curves C and D shows that this contribution does not destroy the significance of observations taken at high turbidities. Accordingly, no corrections for spurious scattering have been attempted.

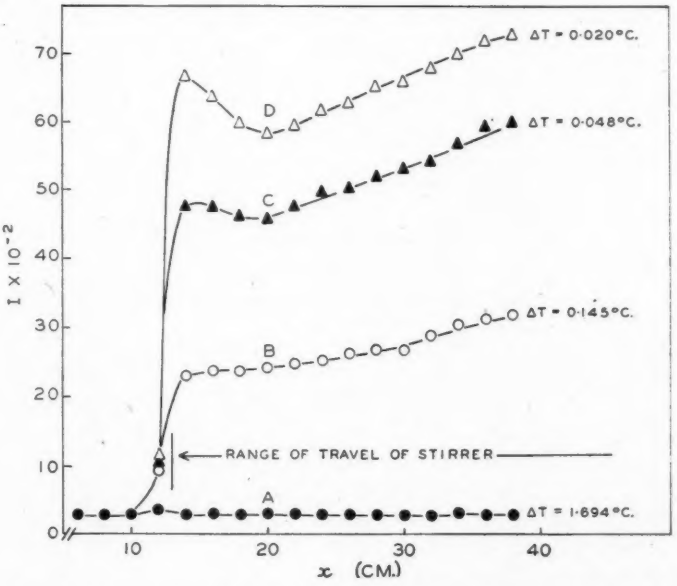


FIG. 3. Variation of scattering coefficient with distance (x) from top of bomb at various temperatures (above critical) in a partially stirred bomb. Note abrupt decrease near upper level of limit of stirrer travel. Scattered intensity is expressed arbitrarily in units of galvanometer deflection.

The data of Fig. 3 were obtained using a bomb 53 cm. long. Here the travel of the stirrer was limited by the mechanical arrangement to the space between the bottom of the bomb and a level 13 cm. from the top. Using a critical filling, the system was heated to $\Delta T = (T - T_c) = 1.7°C$. It was held at this temperature for two hours with continuous stirring for the first hour. The temperature was then lowered to the points indicated in Fig. 3 and observations of the scattering gradient were recorded. It is seen that a marked discontinuity existed at the point of highest stirrer travel at all values of ΔT. This showed the importance of stirring the entire contents of the bomb. Subsequent measurements were made using bombs which could be stirred over the entire length.

Observations of condensation phenomena in another retrograde system showed strong evidence of density gradients. The segment of the equilibrium coexistence curve is shown in Fig. 4. The point designated $\bar{\rho}_m$ is the mean

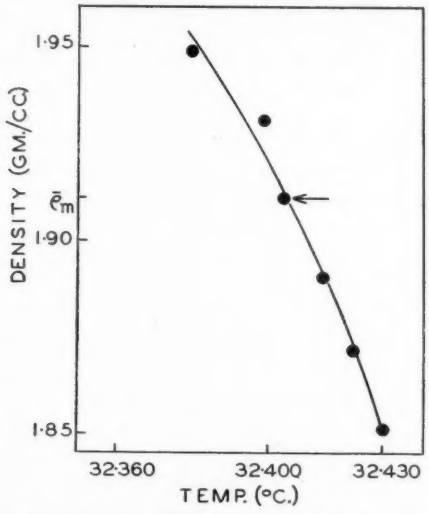


FIG. 4. Segment of the coexistence curve of a retrograde system. The density marked $\bar{\rho}_m$ is that at which maximum scattering occurred.

filling density showing maximum visible opalescence. This curve was determined by cooling (after stirring) at 0.01°C. per hr. at constant densities and observing the temperature of first condensation.

Over the range of $\bar{\rho}$ shown, the "wet fog" of condensation always appeared in the very top of the bomb when the system was cooled. If a density gradient exists, this behavior is expected from the shape of the retrograde coexistence curve (Fig. 4), since the portion of lowest density must be the first to condense on cooling.

Variation of Scattering with Density

A filling showing no retrograde effect was found to have a $T_c = 32.23 \pm 0.02°C.$; this is good agreement with other reported values (1, 8). The temper-

ature at which the meniscus appeared within the confines of the bomb, on cooling at 0.01°C. per hr., without stirring (but with continuous stirring before cooling was begun), was constant within ± 0.001°C. on the Beckmann over a period of several days. The corresponding range of $\Delta\bar{\rho} = (\bar{\rho} - \bar{\rho}_m)$ was − 0.011 to + 0.010 gm. per cc. This represented the operating limits of the injector and corresponded to a range of critical filling densities over which the coexistence curve had a flat top. At the lowest density, the first fog of condensation formed near the bottom of the bomb. As $\bar{\rho}$ was increased, the level of this "wet fog" formation moved upwards until at the highest density attainable, it formed near the top.

Observations of the scattering gradient were made at various values of ΔT and $\bar{\rho}$. At high values of ΔT, the significance of variations of I with $\bar{\rho}$ and x

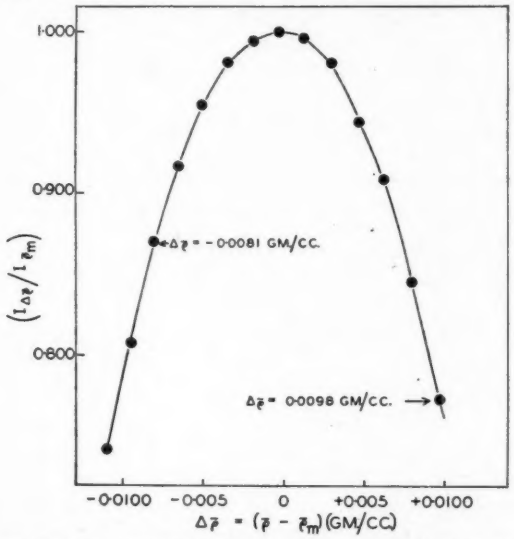


FIG. 5. The variation of scattered intensity with average density at $\Delta T = 0.063$°C. Measurements were made at a level $x = 24.5$ cm.

was reduced by wall scattering. At low values of ΔT (of the order of 0.03°C.) where the turbidity was very high, attenuation of the incident beam by the medium itself reduced the sensitivity. The measurements of $\Delta T = 0.063$°C. are presented as those suffering least from the secondary effects.

The variation of scattered intensity with $\bar{\rho}$ is shown at this temperature in Fig. 5, where the ordinates represent the ratio $I\bar{\rho}/I\bar{\rho}_m$ and $\bar{\rho}_m$ (0.197 ± 0.006 gm. per cc.) is the density at which maximum scattering occurred. Owing to slow drifts in the response of the photomultiplier tube, there is no significant relation between galvanometer readings obtained on different days. For this reason, the ordinates are ratios of readings obtained over a short time interval. The observations (Fig. 5) were taken at $x = 24.5$ cm. from the top of the bomb. Before any readings were taken, the system was allowed to come to an

equilibrium distribution at the lowest density. The system was then slowly compressed in mean density increments of 0.016 gm. per cc. and observations recorded when I became constant. This required about five minutes.

Variation of Scattering with Height

Fig. 6 shows the variation of I (expressed as the ratio $I_{x\text{cm.}}/I_{3.5\text{cm.}}$) with height at values of the mean density corresponding to $\Delta\bar{\rho} = -0.0081$ gm. per cc. and $\Delta\bar{\rho} = +0.0098$ gm. per cc. These observations were made after stirring at $\Delta T = 2°$C. and then cooling (without stirring) to $\Delta T = 0.063°$C. Approximately one hour was required for the gradient to attain a value which showed no change during a further 45 min. period.

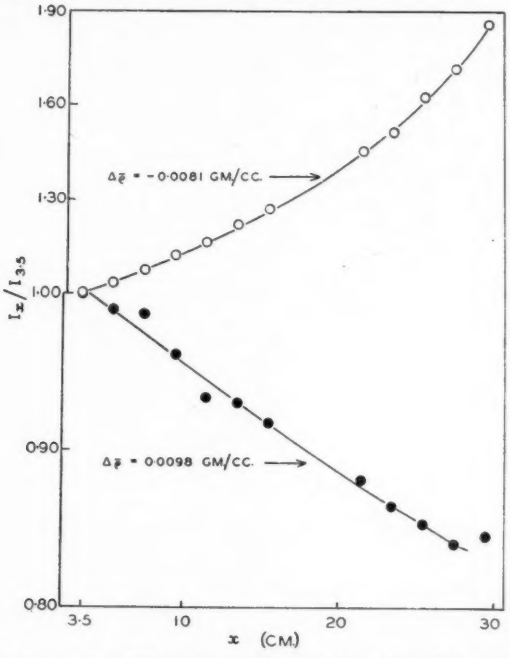


FIG. 6. The variation of equilibrium scattered intensity with height in the bomb at $\Delta T = 0.063°$C.

The slope of each curve in Fig. 6 yields the value $(1/I_{3.5})(\partial I/\partial x)_T$, which is positive at $\Delta\bar{\rho} = -0.0081$ gm. per cc. (upper curve) and negative at $\Delta\bar{\rho} = +0.0098$ gm. per cc. (lower curve). From Fig. 5, at the density $\Delta\bar{\rho} = -0.0081$ gm. per cc., the slope $(1/I_{\bar{\rho}_m})(\partial I/\partial\rho)_T$ is positive, and at $\Delta\bar{\rho} = +0.0098$ gm. per cc., the slope is negative. If the slopes of the two curves at $x = 24.5$ cm. are combined, then the density gradient,

$$\left(\frac{\partial\rho}{\partial x}\right)_T = \frac{(\partial I/\partial x)_T}{(\partial I/\partial\rho)_T},$$

is seen to be positive for both values of $\Delta\bar{\rho}$.

At $\Delta\bar{\rho} = 0$, no maximum in the gradient was resolvable at $\Delta T = 0.063$°C. However, at $\Delta T = 0.020$°C., a marked maximum in I could be seen (visually) by transmitted light near the center of the bomb. Owing to secondary scattering, however, the gradient could not be measured.

The density gradients at the level 24.5 cm. and $T - T_c = 0.063$°C. ($\rho_m = 0.197 \pm 0.006$ gm. per cc.) for the two fillings calculated directly from $(\partial I/\partial\bar{\rho})_T$ (Fig. 5) and $(\partial I/\partial x)_T$ are given below. The corresponding values of β calculated from Equation (2) are given also.

$\bar{\rho}$, gm./cc.	0.189	0.207
$\Delta\bar{\rho} = \bar{\rho} - \bar{\rho}_m$, gm./cc.	−0.0081	+0.0098
$\partial\rho/\partial x$, gm./cm.^{4}	6.2×10^{-4}	1.3×10^{-4}
$(100/\rho)\ (\rho/x)$, %/cm.	0.33	0.062
β, atmos.$^{-1}$	18	3.0

Time Lag after Stirring

In addition to the time lag observed on cooling without stirring as noted above, it was found that when the system was thermostatted and allowed to come to an equilibrium condition at $\Delta T > 0$, and then stirred, appreciable times were required to re-establish equilibrium in the scattering gradient of the quiescent system. Fig. 7 shows the scattering gradient at various times after stirring and indicates a gradual recovery to the equilibrium values previously

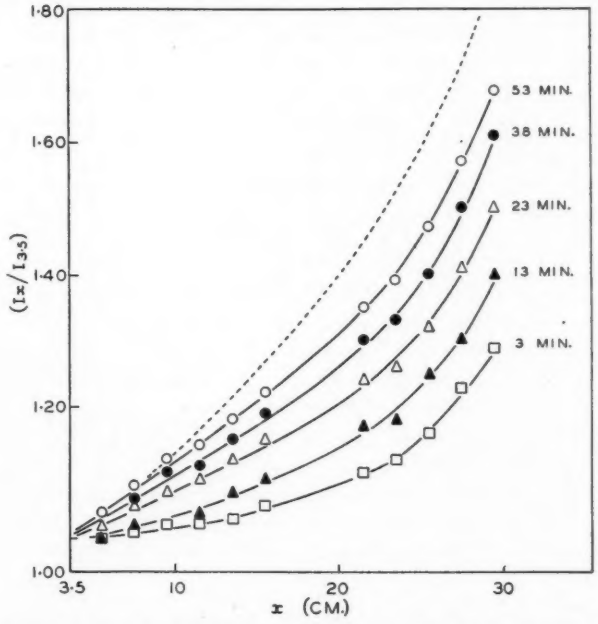


FIG. 7. Scattering gradients observed at various indicated times after stirring at $\Delta T = 0.063$°C. and $\Delta\bar{\rho} = -0.0081$ gm. per cc. The broken line represents the gradient observed on cooling without stirring (Fig. 6) and is taken to be the equilibrium rating.

found. The data shown refer to the system of density $\Delta\bar{\rho} = -0.0081$ gm. per cc. Similar behavior was observed at the higher density $\Delta\bar{\rho} = +0.0098$ gm. per cc.

DISCUSSION

The foregoing observations provide clear evidence of the gravity effect in systems which are in true equilibrium above T_c. The density gradients observed here are not to be confused with the much larger and discontinuous gradients of density (and many other physical properties) which occur in systems heated above T_c *without stirring* (6); in such cases the systems are not in true equilibrium (2, 8) and the discontinuities will probably disappear if the system is efficiently stirred or if sufficient time elapses. It is probable that the behavior of the partially stirred system (Fig. 3) is a related effect.

The observed positions of initial condensation in both the retrograde (impure) and pure systems in themselves indicate an appreciable gravity effect. This was pointed out earlier for the retrograde system. The flat portion of the coexistence curve of the pure ethane (observed over a 10% range in average filling density, a limit imposed by the capacity of the injector) can be reconciled with the concept of a unique critical density (ρ_c) when gravitational compression is taken into account. When a bomb having a critical filling is cooled, initial condensation occurs at the level of ρ_c. As $\bar{\rho}$ increases, this level moves progressively up the bomb as a consequence of the gravity effect. Atack and Schneider (2) noted somewhat similar behavior in the movement of initial fog formation with sulphur hexafluoride but found that the coexistence curve over a range of critical fillings had a rounded top. Recent experiments in this laboratory (17) lead us to believe that Atack and Schneider's system was retrograde, i.e., it contained impurities.

Brief reference should be made to the time lags in establishing the gradient following stirring. It might be expected that the equilibrium gradient would be established as soon as the motion of the gas is damped out. It was not, of course, possible to observe convection currents, but it is unlikely that any would persist for periods as long as one hour. A possible explanation is that the high-velocity ejection of the gas through the hole of the moving plug (Fig. 1*a*) causes a perturbation of β which may have an appreciable relaxation time. This phenomenon may be related to the high sound absorption coefficient in the critical region observed by Schneider (14).

The light scattering method employed here was subject to a number of defects, chief among which was the lower limit of ΔT imposed by secondary scattering. It is hoped to overcome these by improvements in the optical system, and thereby to improve and extend gradient measurements, and to throw further light on the significance of the time lags noted here.

ACKNOWLEDGMENT

The authors are indebted to Dr. W. G. Schneider of the National Research Council for details of design and assistance in the construction of the injector unit.

REFERENCES

1. ATACK, D. and SCHNEIDER, W. G. J. Phys. & Colloid Chem. 54: 1323. 1950.
2. ATACK, D. and SCHNEIDER, W. G. J. Phys. & Colloid Chem. 55: 532. 1951.
3. BLOSSER, L. and DRICKAMER, H. G. J. Chem. Phys. 19: 1244. 1951.
4. HADOW, H. J., SHEFFER, H., and HYDE, J. C. Can. J. Research, B, 27: 791. 1949.
5. KLEIN, M. J. and TISZA, L. Phys. Rev. 76: 1861. 1949.
6. MAASS, O. Chem. Revs. 23: 17. 1938.
7. MACCORMACK, K. E. and SCHNEIDER, W. G. Can. J. Chem. 29: 699. 1951.
8. MASON, S. G., NALDRETT, S. N., and MAASS, O. Can. J. Research, B, 18: 103. 1940.
9. MAYER, J. E. J. Chem. Phys. 19: 1024. 1951.
10. ORNSTEIN, L. S. and ZERNIKE, F. Proc. Acad. Sci. Amsterdam, 17: 793. 1914.
11. OSTER, G. Chem. Revs. 43: 319. 1948.
12. RICE, O. K. J. Chem. Phys. 15: 314. 1947.
13. RUEDY, R. Can. J. Research, B, 9: 637. 1933.
14. SCHNEIDER, W. G. Can. J. Chem. 29: 243. 1951.
15. SCHRAGE, S. Ph.D. Thesis, McGill University, Montreal, Que. 1951.
16. WEINBERGER, M. A. and SCHNEIDER, W. G. Private Communication.
17. WHITEWAY, S. G. and MASON, S. G. Unpublished data.
18. ZIMM, BRUNO H. J. Chem. Phys. 19: 1019. 1951.

CANADIAN JOURNAL OF CHEMISTRY

Notice to Contributors

GENERAL: Manuscripts should be typewritten, double spaced, and the **original and one extra copy** submitted. Style, arrangement, spelling, and abbreviations should conform to the usage of this Journal. Names of all simple compounds, rather than their formulas, should be used in the text. Greek letters or unusual signs should be written plainly or explained by marginal notes. Superscripts and subscripts must be legible and carefully placed. Manuscripts should be carefully checked before being submitted, to reduce the need for changes after the type has been set. If authors require changes to be made after the type is set, they will be charged for changes that are considered to be excessive. **All pages, whether text, figures, or tables, should be numbered.**

ABSTRACT: **An abstract of not more than about 200 words, indicating the scope of the work and the principal findings, is required.**

ILLUSTRATIONS:

(i) *Line Drawings:* All lines should be of sufficient thickness to reproduce well. Drawings should be carefully made with India ink on white drawing paper, blue tracing linen, or co-ordinate paper **ruled in blue only**; any co-ordinate lines that are to appear in the reproduction should be ruled in black ink. Paper ruled in **green, yellow, or red should not be used** unless it is desired to have all the co-ordinate lines show. Lettering and numerals should be neatly done in India ink preferably with a stencil (**do not use typewriting**) and be of such size that they will be legible and not less than one millimeter in height when reproduced in a cut three inches wide. All experimental points should be carefully drawn with instruments. Illustrations need not be more than two or three times the size of the desired reproduction, but the ratio of height to width should conform with that of the type page. **The original drawings and one set of small but clear photographic copies are to be submitted.**

(ii) *Photographs:* Prints should be made on glossy paper, with strong contrasts; they should be trimmed to remove all extraneous material so that essential features only are shown. Photographs should be submitted **in duplicate**; if they are to be reproduced in groups, one set should be so arranged and mounted on cardboard with rubber cement; the duplicate set should be unmounted.

(iii) *General:* **The author's name, title of paper, and figure number should be written in the lower left hand corner (outside the illustration proper) of the sheets on which the illustrations appear.** Captions should not be written on the illustrations, but typed together at the end of the manuscript. All figures (including each figure of the plates) should be numbered consecutively from 1 up (arabic numerals). **Each figure should be referred to in the text.** If authors desire to alter a cut, they will be charged for the new cut.

TABLES: Each table should be typed on a separate sheet. Titles should be given for all tables, which should be numbered in Roman numerals. Column heads should be brief and textual matter in tables confined to a minimum. **Each table should be referred to in the text.**

REFERENCES: **These should be listed alphabetically by authors' names, numbered in that order, and placed at the end of the paper.** The form of literature citation should be that used in this Journal. **Titles of papers should not be given.** The first page only of the references cited should be given. **All citations should be checked with the original articles.** Each citation should be referred to in the text by means of the key number.

REPRINTS: A total of 50 reprints of each paper without covers are supplied free to the authors. Additional reprints will be supplied according to a prescribed schedule of charges. On request, covers can be supplied at cost.

Approximate charges for reprints may be calculated from the number of printed pages, obtained by multiplying by 0.6 the number of manuscript pages (double-spaced typewritten sheets, 8½ in. by 11 in.) and making allowance for space occupied by line drawings and halftones (not inserts). The cost per page is tabulated at the back of the reprint request form sent with the galley.

Contents

Page

The Reactions of Acetaldehyde with Methanol—*G. W. Meadows and B. deB Darwent* - - - - - - - - - - - - 501

Effect of Addition Agents on Copper Deposition in a Convection-free System—*R. C. Turner and C. A. Winkler* - - - - - 507

Purification and Composition of a Polyuronide Hemicellulose Isolated from Wheat Straw—*G. A. Adams and A. E. Castagne* 515

The Estimation of Free Amino Acids Using a Microdiffusion Technique—*W. B. McConnell* - - - - - - - - - - 522

The Decarboxylation of Anthranilic Acid—*W. H. Stevens, J. M. Pepper, and M. Lounsbury* - - - - - - - - - - 529

The Methoxymercuration of 2-Methyl-1-phenylpropene-1—*L. Berman, R. H. Hall, R. G. Pyke, and George F Wright* - - 541

Coexistence Phenomena in the Critical Region. I. The Gravity Effect in Ethane from Light Scattering—*F. E. Murray and S. G. Mason* - - - - - - - - - - - - - - - 550

Printed in Canada *by* University of Toronto Press